DECEMBER, 1960
Vol. 14, No. 12

# FT food technology

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1961 . . . . . ?

Nicholas Appert
1961 . . . . . ?

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Industrial Achievement
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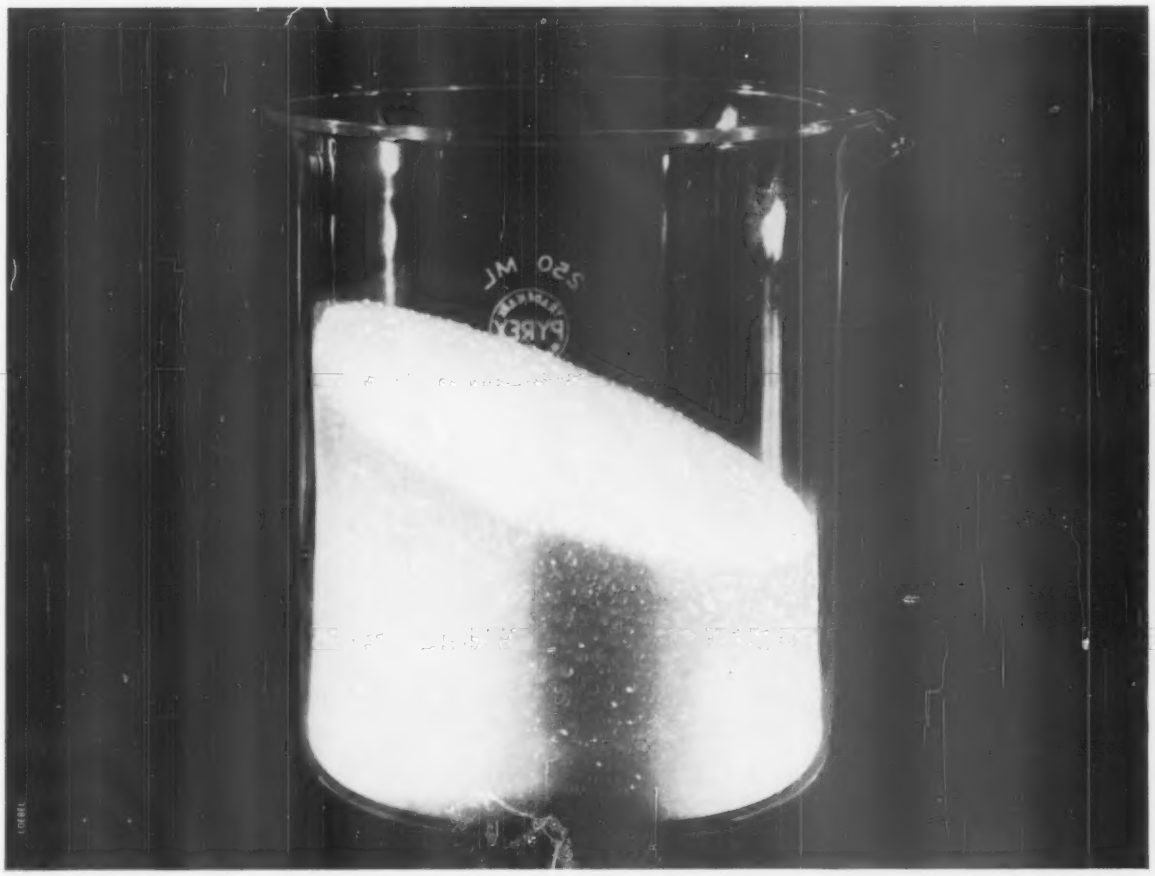


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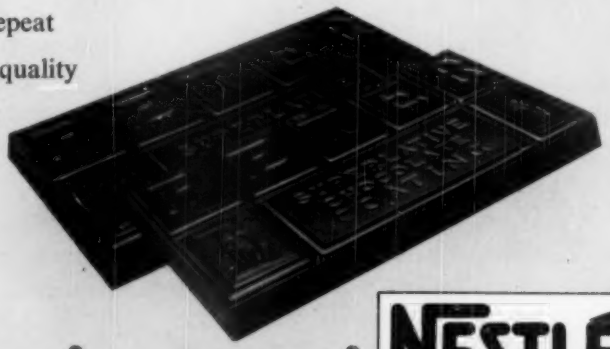

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# FT food technology

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**Volume 14, Number 12**

PUBLISHED MONTHLY BY THE INSTITUTE OF FOOD TECHNOLOGISTS


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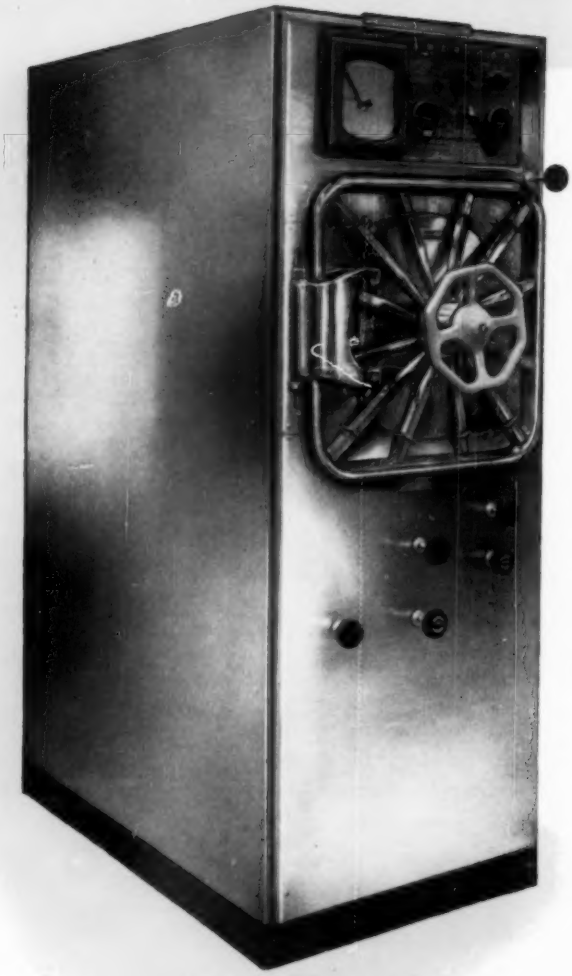

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## Dr. George F. Stewart to Become Executive Editor of IFT Journals, on January 1, 1961

On January 1, 1961, in accordance with decisions made by the Council at its May 1960 meeting, Dr. George F. Stewart will become Executive Editor of the IFT journals. Dr. Stewart, Chairman of the Department of Food Science and Technology, University of California, Davis, brings to his new post credentials in three fields—science, education, and editorial work. As co-editor with Dr. Emil Mrak of the distinguished series ADVANCES IN FOOD RESEARCH he has come into close contact with the major work being done in food research, the people involved, and the problems of greatest concern.

Dr. Stewart's professional activities include many years of participation in the work of the Institute of Food Technologists, of which he is a charter member, and in that of other organizations in the food field. Among these are the Poultry Science Association and the American Dairy Science Association. He has also contributed to the work of the American Public Health Association and the American Association for the Advancement of Science.

Dr. Stewart received his B.S. (Chemistry) from the University of Chicago and his Ph.D. (Biochemistry) from Cornell University. Among numerous honors bestowed upon him mention should be made of the Christie Award, given for outstanding poultry products technology research, and a Fulbright Fellowship (Australia).

Dr. George F. Stewart

Dr. Stewart spent the first five years of his professional career in industry (Ocoma Foods, Inc., Omaha) but since 1938 he has been engaged in university teaching, research, and administrative work—first at Iowa State University (1938–1951) and then at the University of California, Davis.

A more detailed announcement concerning the change in the editorship will be published in the January issue of Food Technology. The purpose of the present brief item is to extend welcome to the new editor, Dr. Stewart, his able assistant, Mrs. Stewart—and to notify authors that the new address of the Editorial Office is P. O. Box 164, Davis, California.

---

## *Call for Nominations*—Food Technology Achievement Award

The IFT Food Technology Achievement Award is given annually to recognize and honor an outstanding food process and/or product, which represents a significant advance in the application of Food Technology to food production, and which has been successfully applied in actual commercial operation for at least six months but not over four (4) years prior to the date of calling for nominations. Insofar as possible, this Award shall be personalized by public recognition of the individuals(s) responsible for the achievement.

The Award will include the following: to the Company or Institution, a bronze Plaque. To the individual(s) judged to have made major contributions to the achievement, either through basic research or development, public recognition at the time of the presentation of the Award, and an engrossed Plaque.

Nominations for this Award may be made by any member of the Institute of Food Technologists. Each member of the Institute is urged to give serious consideration to the nomination of a product or process, not necessarily one in which your company is concerned. Each nomination must be in letter form and include the following:

A. Name of Company or Institution.
B. Name of Product and/or Process.
C. Description of Product or Process.
D. Statement of reason for considering this a meritorious achievement.
E. Statement listing individuals chiefly responsible for the achievement and their individual contributions.
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# CALL FOR NOMINATIONS

## The Babcock-Hart Award

Any member of the Institute of Food Technologists may submit a nomination for the 1961 Babcock-Hart Award between now and the January 1, 1961 deadline.

**Nominations must be made on the prescribed nomination form which may be obtained from the Executive Secretary, in order to be considered.**

Donor of the $1,000.00 and engrossed plaque accompanying the Award is the Nutrition Foundation. Purpose of the Award is: "To honor a person who has distinguished himself or herself by contributions to Food Technology which have resulted in improved public health through some aspect of nutrition or more nutritious food."

A nominee, to be eligible, must have made a technological development, or a scientific contribution leading directly to a technological development, which favorably affects the nutritional well-being of the public. **Contributions in clinical nutrition and fundamental scientific studies in nutrition cannot be considered as qualifying according to conditions established by the Donor.**

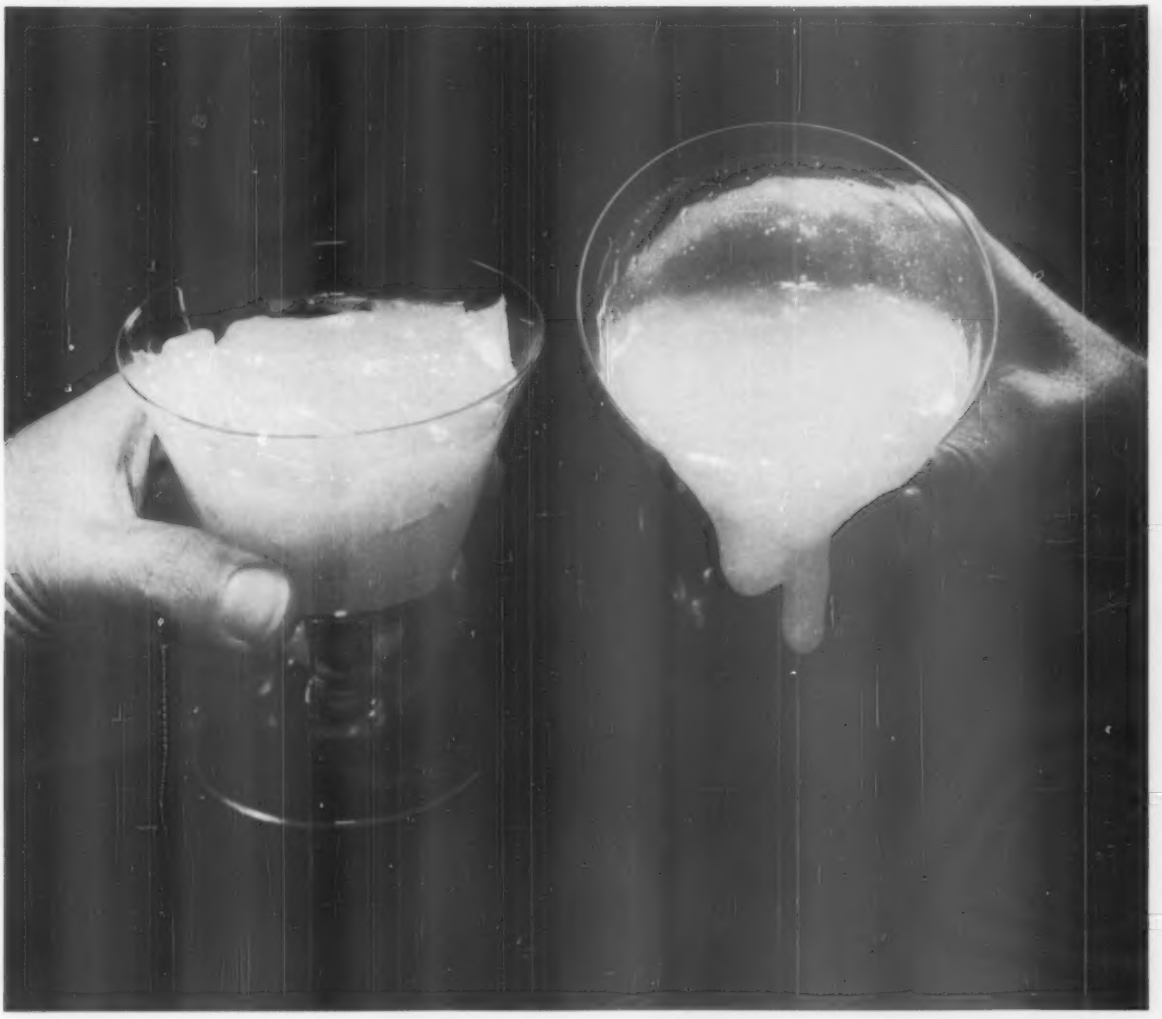
## The Nicholas Appert Award

Any member of the Institute of Food Technologists may submit a nomination for the 1961 Nicholas Appert Award between now and the January 1, 1961 deadline.

**Nominations must be made on the prescribed nomination form which may be obtained from the Executive Secretary, in order to be considered.**

Donor of the $1,000.00 honorarium accompanying the Award is the Institute of Food Technologists. The Award, originated by the Chicago Section, also includes a Bronze Medal which is donated by that Section. Purpose of the Award is: "To honor a person for pre-eminence in and contributions to the field of Food Technology."

Qualifying criterion for a nominee includes merely the fact that the nominator consider the individual so pre-eminent in the field of Food Technology that he or she should be acclaimed for contributions thereto. This precludes consideration of personality or other characteristics outside the field of contributions to Food Technology, as these factors may not be considered by the Awards Jury in making the selection.



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# TECHNICAL ARTICLES IN BRIEF

*Evaluation of methods of care* — *Frying life of fat*

**The effect of method of care on the frying life of fat.** M. Elizabeth Rust and Dorothy L. Harrison.

The effect of 4 methods of care on the frying life of a hydrogenated vegetable fat processed for food service deep fat drying was investigated. The methods were: I—filtered, stored in a covered metal container at 2° to 5° C, and 20% (of original weight) fresh fat added each 8-hr frying period; II—filtered, stored in a cleaned fryer at 20 to 25° C, and 20% fresh fat added each frying period; III—filtered, stored in a cleaned fryer at 20 to 25° C and fresh fat added to replace that lost during each 8-hr frying period; IV—skimmed, stored in fryer at 20 to 25° C, and fresh fat added to replace that lost at each frying period. Frying life of the fat was determined by palatability scores for French fried potatoes. Fat cared for by Methods I and II had a frying life of more than twice that of fat cared for by Methods III and IV. Methods I and II also required approximately twice as much fat as Methods III and IV to fry 100 g of potatoes, which would affect the cost of a serving of French fried potatoes. The cost of a 100% fat turnover that would be required more than twice as often for Methods III and IV than for Methods I and II should be considered. Filtering and refrigerating the fat and cleaning the fryer between frying periods also prolonged the frying life of the fat. (See page 605).


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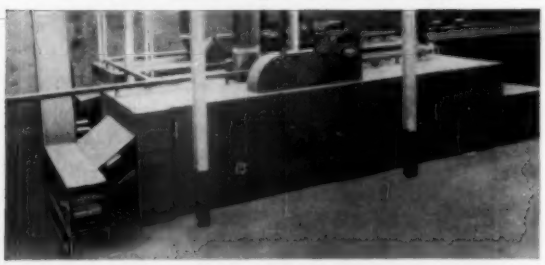

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*Antibiotic* — *Preservation of poultry*

**The effectiveness of Acronize chlortetracycline in poultry preservation following long term commercial use.** A. Abbey, A. R. Kohler, and P. F. Hopper.

Acronize remained effective (75% average shelf-life extension) in the retardation of poultry spoilage after 5 years of continued commercial use. Acronized poultry held under ice demonstrated an increase (48% average) in shelf-life over control poultry. When poultry was stored in polyethylene bags, an increase (77% average) in shelf-life over the control is noted. The indications are that an increase of insensitive microorganisms in a developing spoilage population on Acronized poultry is most likely caused by "selection" rather than "development." (See page 609).

*Flavor evaluation* — *Effect of number of judgments*

**The effect of number of judgments in a test on flavor evaluations for preference.** Lois A. Sather and Lyle D. Calvin.

Four products—canned peaches, hamburger, tomato juice, and green beans—were scored for preference by a panel of 175 to 191 untrained flavor judges. The 20 samples of each product were assigned to four trays and judged in one continuous test period. The order of presentation to the judges was assigned so as to have each tray tested in each possible order an equal number of times. It was assumed that the preference differences for the samples on a tray were flavor differences. Results on tomato juice indicated the judges had a lower ability to detect differences among samples on the first tray than when the same samples were served on succeeding trays. On the other three products tested, (green beans, hamburger, and peaches), tray order did not significantly affect the ability of the judges to discriminate between samples. This indicates that for mild products, such as included in this test, up to 20 samples may be included in one test period with no decrease in the

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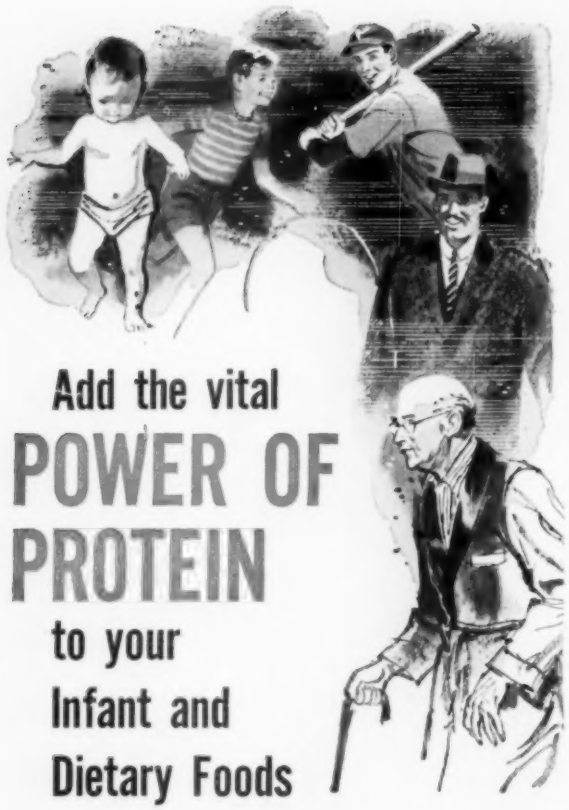

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judges' ability to discriminate flavor preferences among samples. (See page 613).

*Dehydration method* *Applied to English walnuts*

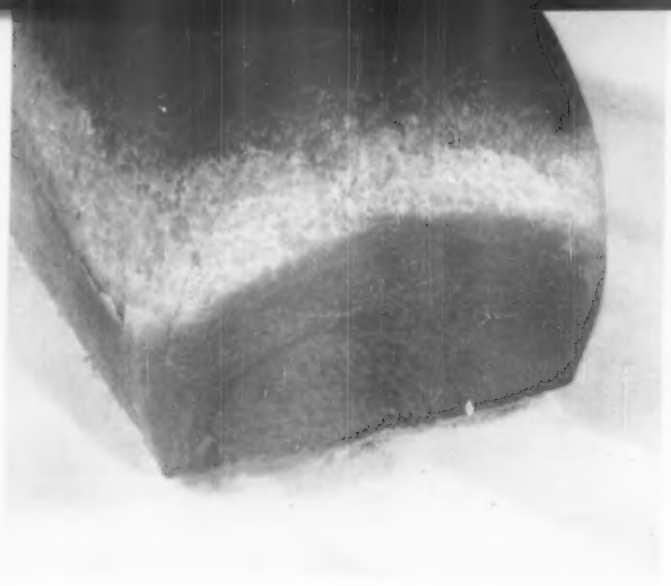
**Studies on English (Persian) walnuts, Juglans regia. II. Dehydration of kernels with the belt-trough dryer.** Louis B. Rockland, E. Lowe, D. M. Swarthout and R. A. Johnson.

Dehydration rates have been estimated for high-moisture walnut kernels in a laboratory model belt-trough dryer at temperatures ranging from 160° to 255° F. Kernel moistures were reduced to the optimum moisture range (3.5 ± 0.3%) from 15 to 20% within 65 minutes during single-stage drying at 190° F and within 50 minutes during two-stage drying for about equal periods at 180° and 210° F, respectively. Under these conditions no significant off-flavors developed during dehydration. However, excessive drying to 3.2% moisture or lower produced a slightly toasted flavor and more rapid deterioration, especially at the higher dehydration temperatures. At an air inlet temperature of 255° F kernels developed a distinct toasted flavor within 15 minutes although the kernel moisture level was above 5%. These investigations corroborate Batchelor and Christie's observations that the critical temperature for walnuts decreases with increasing moisture content. These investigators also observed significant flavor changes or accelerated rancidity development for in-shell walnuts after bin-drying for 24 to 48 hours at 120° F and higher. However, the present authors have observed no initial impairment of kernel quality at drying temperatures as high as 210° F and kernel temperatures up to 145° F after the short drying periods required by the belt-trough dryer. Kernels dried on the belt-trough dryer developed a slight polish although kernel abrasion appeared to be minimized. Improved kernel appearance may have been due in part to removal of loose oil-soaked pellicle, chaff and fines which were removed by updraft in the warm air stream penetrating the kernel bed. No significant loss of fines or clogging of the screen belt was observed. (See page 615).

*Maturity determination* *Size of sample*

**Size of sample for maturity determination of canning corn.** H. B. Cannon, E. A. Asselbergs, and W. P. Mohr.

This study represents an attempt to devise suitable sampling procedures for determining the mean moisture content of a field of corn using the Steinlite method of determination. Two factors influencing variability within the field have been estimated and have shown that position in the field was considerably more important than ear to ear variation (including determination to determination variability, etc.). Using these estimates the accuracy, expressed as confidence limits, for a number of different schemes has been presented. The investigation has shown that very inaccurate estimates of the mean moisture content of a field of corn may result when an estimate is based upon too few samples. Increasing the number of positions in the field at which ears are harvested appears to be much more efficient in improving accuracy than increasing the number of ears at any one position. While it is recognized that these results apply to these specific data, the main considerations may be useful in deciding upon a sampling scheme under all ordinary conditions. (See page 619).



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*Radiation preservation* — *Cured ham*

**Radiation resistance of the natural bacterial flora of cured ham.** W. L. Brown, C. Vinton, and C. E. Gross.

Three separate experiments were conducted to study the radiation resistance of the natural bacterial flora of canned ham. Most strains were extremely sensitive to irradiation and were destroyed at low levels. Two strains of cocci were able to survive high levels of radiation but were not studied extensively since in commercial processing they would be destroyed with low levels of heat. Small numbers of spores were able to survive high levels of radiation in cans of cured ham. These spores were identified as the S-2 strain of PA 3679. If curing salts (salt, sugar, nitrate and nitrite) were present in normal amounts the ground ham did not spoil in the cans given at least 0.5 megarad irradiation when incubated at 26°C and 30° C for a period of two months. The spores were able to grow and produce spoilage through a radiation level of one megarad, provided no curing salts were present in the meat. Counts after incubation indicated that the cells were increasing in numbers in cans of meat containing no curing salts. No marked differences were found in destruction of bacteria between a gamma source and electron beam source of irradiation. (See page 622).

*Anthocyanins* — *Effect on growth of microorganisms*

**Anthocyanins. II. Action of anthocyanin pigments and related compounds on the growth of certain microorganisms.** John J. Powers, Dardjo Somaatmadja, Dan E. Pratt, and Mostafa K. Hamdy.

Pelargonidin-3-monoglucoside and delphinidin-3-monoglucoside inhibited the growth of *E. coli*. Malvidin-3-5-diglucoside stimulated growth at some stages, inhibited it at others. Delphinidin and malvidin stimulated growth. The compounds, 5-desoxy-3′-methoxy-apigeninidin-chloride-4′-methyl-ether, apigeninidin-chloride, 5-desoxy-apigeninidin-chloride-4′-methyl-ether, and pelargonidin-3-monoglucoside, each inhibited growth of *S. aureus*. Delphinidin-3-monoglucoside, malvidin 3,5-diglucoside, malvidin-3-monoglucoside, and delphinidin accelerated multiplication, evidenced by increased growth around the sensitivity discs. *L. casei* generally was stimulated by malvidin-3-5-diglucoside and malvidin. At a concentration of 10 mg pigment per disc, malvidin-3,5-diglucoside inhibited growth; pelargonidin-3-monoglucoside likewise was inhibitory. Heating of synthetic compounds resulted in loss of activity in some cases and in gaining activity in cases where the non-heated compound had been without activity. The $R_f$ values of the compounds and the infrared spectra of some of the compounds were determined. Liberation of phloroglucinol in acid solution during the heating of the synthetic compounds was demonstrated by paper chromatographic analysis. (See page 626).

*Sporicidal effect of chlorine* — *B. coagulans*

**Effect of chlorine on spores of Bacillus coagulans.** T. R. LaBree, M. L. Fields, and N. W. Desrosier.

The sporicidal effect of chlorine on the spores of *Bacillus coagulans* was influenced by pH, temperature, and chlorine concentration. By increasing the temperature to 60° C (140° F), the sporicidal effect was in the range of practical use in a cannery. The amount of chlorine lost, when 60° C was used, was considered insignificant. The sporicidal effect was also increased to a practical point when the chlorine content was increased to 20 ppm. (See page 632).

*Ripeness level of tomatoes* — *Effect on canned juice*

**Effect of ripeness level on consistency of canned tomato juice.** B. S. Luh, Fidel Villarreal, S. J. Leonard, and M. Yamaguchi.

Tomato juices were canned during the early, middle, and late seasons at four different stages of ripeness as judged by skin color and the Agtron E colorimeter. The effect of ripeness level on pectin, cellulose, and consistency of canned juices was investigated. Total pectin decreased with ripening. Protopectin decreased in proportion to an increase in water-soluble pectin, indicating solubilization of protopectin as the fruit matured. Pear-shaped tomato varieties contained higher pectin than round-shaped varieties. The varietal characteristics of resistance to *Fusarium* and *Verticillium* wilt appeared to be independent of pectin content. The Brookfield viscometer and the pipette were used to determine consistency. Juices made from firm-ripe tomatoes were thicker in consistency than those made from canning-ripe or soft-ripe tomatoes. Consistency was lowered when the pulp was depectinized with versene and then recombined with the serum. This indicates that pectic substance in the tomato pulp is an important contributor to juice consistency. Various factors influencing tomato juice consistency are discussed. (See page 635).

*Causes of breakdown* — *Frozen sliced strawberries*

**Factors contributing to the breakdown of frozen sliced strawberries.** William A. Sistrunk, Robert F. Cain, Edward K. Vaughan, and Harry B. Lagerstedt.

A study was made to assess the importance of variety, date of harvest, mold inhibitor spray, holding time and temperature after harvest, rate of freezing, method of slicing, and length of time of mixing fruit and sugar in relation to the breakdown in character of frozen sliced strawberries. The four varieties—Northwest, Marshall, Siletz and Puget Beauty—were found to react differently to freezing although fresh fruit quality was maximal. Percentages of mushy slices in the frozen product were lower in the Northwest variety. Also, shear-press measurements of firmness were higher than in the other varieties. Wherever harvest was involved, there was an increase in the per cent of mushy slices and water-soluble pectin content in the frozen product that was prepared from fruit harvested beyond mid-season. Furthermore, there was a decrease in the viscosity of syrup and shear-press measurements of firmness of the thawed frozen product. Maturity probably had a great deal to do with the effect of date of harvest. An increase in mixing time beyond the time necessary to dissolve the sugar significantly increased the per cent of mushy slices and syrup viscosity of the thawed frozen slices of Siletz variety. Shear-press measurement of firmness was decreased. In the Siletz variety, there was a steady increase in the per cent of mushy slices when fresh fruit was held either at 34° F. or at room temperature prior to processing. Syrup viscosity decreased during holding up to 24 hours. (See page 640).

*Enzymology* — *Polygalacturonase and softening of brined cherries*

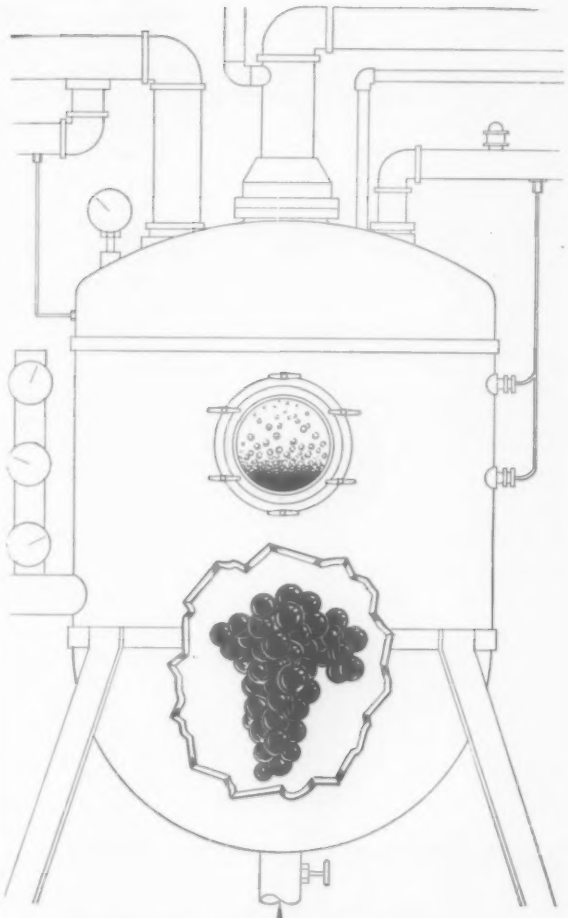
**Inhibition of polygalacturonase in brined cherries.** H. Y. Yang, W. F. Steele, and D. J. Graham.

Polygalacturonase has been found to be responsible for the severe softening of brined cherries by the degrading of pectin in the cherries. The enzyme is inactive at the pH of 1.6 or lower. By using calcium chloride instead of calcium hydroxide as the firming agent in $SO_2$ brine, such a low pH can be obtained. Nevertheless, at this low pH cherries tend to crack and their skin is injured easily. Calcium chloride brine also produces cherries with an undesirable, tough texture and low crispness. This is considered by many maraschino processors to be objectionable. For these reasons, calcium hydroxide is preferred as a firming agent in the cherry brine. The pH of a calcium hydroxide brine is about 2.1 and increases to about 3.0 when cherries are added. At this pH range, polygalacturonase remains active. The enzyme, however, can be inactivated by the addition of 0.01–0.025% of commercial alkyl aryl sulfonate to the brine. Five commercial preparations tested were found to be equally effective as inhibitors of polygalacturonase in $SO_2$ Ca $(OH)_2$ brine. By use of radioactive isotope tracer techniques, 0.6 ppm of residual alkyl aryl sulfonate was found to remain in the leached cherries when 0.01% of a commercial preparation of alkyl aryl sulfonate was added to the original brine. No undesirable effects were observed by the authors on maraschino cherries made from brined cherries treated with as much as 0.1% of commercial alkyl aryl sulfonate. It is concluded that polygalacturonase can be inhibited by a cherry brine with the following formulation: 1.25% sulfur dioxide, 0.7% calcium hydroxide, and 0.02% commercial 40%-active alkyl aryl sulfonate. (See page 644).

*Packaging effect* — *Ripening of mature green tomatoes*

**Effect of packaging films and storage temperatures on the ripening of mature green tomatoes.** John C. Ayres and Lincoln C. Peirce.

Tomatoes stored at 5° C did not ripen normally; apparently this temperature was subminimal and predisposed the fruit to decay. At other temperatures, rate of ripening proceeded in the following order: ambient room >25° >20° >15° >10° C. Tomatoes kept for as long as 2½ months at 10° C and for 2 months at 15° C. The fruits spoiled in less than 1½ months at 20° C and in a few days at 25° C or at ambient temperature. Use of shear press values appears to be of value in assessing stage of maturity (ripeness). Judging panels considered trays of tomatoes overwrapped with cellulose acetate (CA) attractive when compared with unwrapped tomatoes or with fruits packaged with polystyrene (Q641.4), polyethylene, PHD cellophane, Pliofilm, or polyvinylchloride (Saran) and Cryovac. CA had good transparency, adequate mechanical strength, and sufficient permeability to gases and water vapor transmission. However, this film permitted greatest loss in weight of the product. There was some fogging in all of the other films; this defect was most obvious in trays overwrapped with the more impermeable types of packaging films. Not only did fruits rot more quickly in polyethylene, Pliofilm, Saran and Cryovac, but in addition, the tomatoes had less desirable taste. Weight losses were minimal in trays wrapped in Pliofilm, Saran and Cryovac but the fruit decayed and the



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trays became water-logged. Tomatoes did not ripen or color normally when packaged with polyethylene. (See page 648).

*Evaluation* *Dehydrated flavoring materials*

**Instant bread mix: Studies on the dehydration of flavoring materials.** C. O. Chichester, Nancy Sharrah, and Marion Simone.

Freeze, spray, and drum drying techniques were successful in producing dried bread-flavoring materials from cell-free liquid broths. Breads made with these mixtures were ranked slightly different in bread flavor intensity and no different in preference by a laboratory panel. These dried materials maintained sufficient storage stability, from the standpoint of observed appearance, color retention and moisture pick-up over a period of 2 months. (See page 653).

*New product* *Instant bread mix*

**Instant bread mix: consumer evaluation of prepared bread.** Marion Simone, Nancy Sharrah, and C. O. Chichester.

Instant breads, made with freeze dried, spray dried, drum dried, or liquid flavoring preparations, were evaluated by a laboratory and consumer panel. Comparative ratings of bread flavor and preference by the laboratory panel resulted in the following rank order of the samples: liquid, freeze dried, spray dried, and drum dried. The rank order of general likeness reaction by the consumer panel was liquid, drum dried, spray dried, and freeze dried. No significant differences were indicated by either panel among the three dried flavor preparations. An over-all consumer rating of the instant breads was 4.65 (between *like slightly* and *neither like nor dislike* on a 7-level structured scale). There were significant age and sex effects on ratings of the instant bread by the consumer population tested. The younger and older groups and females, rated all samples higher than the middle age groups and males. Bread-eating habits had an apparent effect on the consumer's degree of liking of the instant bread. The significant differences in loaf volume among the 4 samples were closely related to the laboratory panel's comparative ratings of flavor intensity and preference and to the consumer panel's response to textural quality. (See page 657).

*Modification of consistency* *Dairy products*

**Consistency modification of dairy products.** A. R. Handleman.

Phosphates and citrates are used to modify and control the consistencies of milk-based products such as process cheese, instant pudding, and evaporated milk. It was found that dicarboxylic acids such as adipic affect the consistencies of milk protein dispersions. In systems where condensed phosphates have produced gels, adipic acid has reduced viscosity by as much as eightfold. Results of this study suggest interesting possibilities in controlling consistencies of cheese products, cocktail dips, evaporated milk, dairy-based sauces, etc. Since the data were obtained with milk solids, it is probable that some such products could be marketed in the dry form. (See page 662).

*Statistical technique* *Applied to palatability data*

**A note on the application of orthogonal polynomials to data on palatability when the levels of a treatment are unequally spaced.** Jacob N. Eisen.

Orthogonal polynomials were used to evaluate panel scores for flavor of fresh broccoli cooked by boiling for four unequally spaced times: 5, 10, 15, 25 minutes. A quadratic equation, $\hat{Y} = 1.04 + .356X - .013X^2$, was calculated for estimating flavor score from cooking time. This equation was formed by adding several terms to the linear equation. The example demonstrates that, even when the treatment levels (X) are unequally spaced, the goodness of fit of the linear, quadratic and cubic regression curves can be independently and successively tested for significance. (See page 663).

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# REGIONAL NEWS

## JOINT MEETING, CHICAGO AND INDIANA

Just about everything went right on the occasion of the joint meeting of the Chicago-Indiana Sections. Purdue won the football game. The barbecued chicken left absolutely no room for criticism. The rapid sur-

vey of the status of food technology at Purdue, a pre-luncheon program designed to give just the right amount of information on the aims and philosophy, was highly effective. The "lecture" by Professor Hartsell stimulated everyone with the urge to go back to school.

### General Foods Award to Hartung

Theodore E. Hartung, extension poultryman at Colorado State University, was presented the General Foods Scholarship Award at this joint meeting of the Chicago and Indiana Section of the Institute of Food Technologists. The presentation was made on behalf of General Foods and the Institute by George Marshall of the Jello Division.

**Theodore E. Hartung (right) receiving General Foods Scholarship Award from George Marshall.**

The greatly coveted award consists of a fellowship carrying a total stipend of $4000 per annum for Hartung to pursue graduate work toward his Ph.D. degree in any area of food technology at the institution of his choice.

Hartung is currently registered in the Graduate School of Purdue University majoring in poultry products technology under Dr. W. J. Stadelman, professor of poultry science. He is on sabbatical leave from the poultry department of Colorado State where for the past seven years he has ably served the industry as extension poultryman. He and Mrs. Hartung and their two children, Deborah Jean, 8 and Robin Leon, 5 are living at 213–7 Halsey Drive, West Lafayette.

## WASHINGTON

Our Section held its regular monthly meeting at 8:00 P.M. Sept. 28 at the National Canner's Association building. After a short business meeting the

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membership turned its attention to the program of the evening, which was furnished by the National Association of Frozen Food Packers. Their two speakers, Mr. H. P. Schmitt, Research Director, and Mr. L. S. Fenn, Ass't Research Director, discussed the subject "Regulatory Agencies View Frozen Foods."

Mr. Schmitt's paper stated that frozen foods first came under the regulatory microscope in 1956 when the Association of Food and Drug officials of the U. S. (AFDOUS) passed a resolution for the development of a code for frozen food handling conditions and bacteriology. AFDOUS later adopted a handling code, on the zero F. basis, in 1959, which did not include a provision for bacterial counts.

Three types of bacterial counts received considerable discussion and investigation during development of this code. These were the standard plate count, determination of coliforms and determination of coagulase positive staphylococci. Mr. Schmitt stated that the setting up of goals based on these determinations has had a beneficial effect on the industry, with these goals being attained through adherence to four basic rules during manufacture.

Mr. Schmitt next discussed the short-comings of these three bacteriological determinations, particularly the conditions under which they could lead to misleading information, and concluded that the matter of arriving at specific bacterial guide lines was very complex, particularly when statutory implications were considered.

In conclusion Mr. Schmitt stated that the State of Massachusetts had disregarded recommendations of AFDOUS and microbiological experts and had promulgated a regulation establishing bacterial limits on precooked frozen foods. Enforcement has been delayed to February 1, 1961.

Mr. Fenn discussed the effect of storage and transportation on the quality of frozen foods. He stated that research had established the proper means for handling and storing frozen foods. However, isolated incidents of improper handling do occur and better information is needed to inform the parties concerned on the extent of damage to the food. He stated that this problem had been given considerable thought by the NAFFP. A standardized system for inspection and reporting on the condition of frozen products in transit and storage was suggested by the speaker. A tentative score sheet has already been developed for frozen green and wax beans to serve as an example of a proposed verification service.

These papers precipitated a lively discussion, after which the members adjourned for refreshments.

—*A. P. Sidwell*

## PHILADELPHIA

Our October meeting was held at Dairy Maid Restaurant, Germantown. There was an overflow audience of 150. The Philadelphia Section has joined the

(Continued on page 17 following technical section)

# The Effect of Method of Care on the Frying Life of Fat[a, b]

**M. Elizabeth Rust and Dorothy L. Harrison**

Departments of Institutional Management and Foods and Nutrition, Kansas State University, Manhattan, Kansas

(Manuscript received June 27, 1960)

DEEP FAT FRIED FOODS are accepted almost universally. Nevertheless, the susceptibility of frying fats to deteriorative changes and the transfer of undesirable flavors to the food cooked in the degraded fat is a major problem.

Bennion and Hanning *(1)* reviewed several studies related to the changes in fat during frying. Carlin, Hopper, and Rockwood *(2)* discussed three heat induced chemical reactions that take place simultaneously when fats are held at frying temperatures; namely, hydrolysis, polymerization, and oxidation.

The length of time that a fat can be used for frying has been related to changes in the fat during frying *(5)*. Various methods of care for frying fat have been suggested by equipment manufacturers and fat processors. Each of these treatments has purported to lengthen the frying life of the fat, and thus reduce the cost per serving of food. The purpose of this study was to determine the effect of 4 methods of care used in restaurants on the length of the frying life and deteriorative changes in the fat.

## EXPERIMENTAL PROCEDURE

To establish the methods of caring for fat commonly used, a survey of 96 restaurants and food services in northeastern and north central Kansas was made. From the survey information and recommendations of fat processors and fryer manufacturers the 4 methods used in this study were determined. These were:

Method I: At the end of each frying period the fat was drained from the fryer and filtered through 4 thicknesses of cheese cloth. The fat was weighed and 100 g removed for objective tests. Enough additional fat was removed to decrease the weight to 80% of the original. The fat was placed in a metal container, covered, and stored at 2° to 5° C until the next frying period. At the beginning of each subsequent frying period fresh fat equal to 20% of the original weight was added. After each period the fryer was cleaned with a commercial fryer cleaner, rinsed and dried.

Method II: The fat and fryer were treated as described for Method I except that the fat was stored in the cleaned, covered fryer at 20° to 25° C.

Method III: The fat and fryer were handled as in Method II except only enough fat was added at each frying period to replace the fat lost during frying and that removed for objective tests. The quantity of fat added was approximately 7% of the original weight.

Method IV: At the end of each frying period the fat was skimmed to remove the charred particles of potatoes floating on the surface, drained from the fryer, weighed, and 100 g removed for objective tests. The fryer was wiped with a clean dry cloth, and the fat returned to the fryer, covered and stored at 20° to 25° C. At the beginning of each subsequent frying period sufficient fresh fat was added to replace the frying loss plus the 100 g removed for objective testing (approximately 7% of the original weight).

Four automatic electric fryers made by the same manufacturer were used in the study. Two of these fryers each had a capacity of 25 lb of fat and the others, 15 lb each. Preliminary work indicated that for fryers of these capacities, size had no effect on the factors to be investigated if the ratio of the weight of the food cooked to the weight of the fat was the same for each fryer. The methods of caring for the fat were assigned to the fryers at random.

A hydrogenated vegetable fat, processed for food service deep fat frying, was stored at 2° to 5° C until used. All of the fat was from the same batch, and was packaged in 110 lb drums. The acid numbers, determined for each drum of fat just prior to use, were zero.

U. S. Number 1 Idaho Russet potatoes were purchased as needed, and stored at 20° to 25° C for 5 to 7 days prior to use. The potatoes required for each frying period were peeled on a portable abrasive type electric peeler, eyed by hand, and cut with an electric French-fry cutter to a $\frac{3}{8}$-inch cross section.

The weight of raw potatoes cooked at each frying period equaled the initial weight of the fat in a given fryer. The potatoes for each fryer were divided into 8 equal batches. This gave a frying ratio of 1 g of potatoes to 8 g of fat.

One frying period was 8 hr. The fat was pre-heated in the fryers to 200° F (93° C), and held at this temperature for 3 hr before frying was started. Fifteen min were allowed for the temperature of the fat to be raised to 365° F (185° C). Then, each batch of potatoes was cooked 6 min with 10 min between consecutive batches. After $5\frac{1}{4}$ hr, the thermostats on the fryers were re-set to 200° F (93° C) and the fat held at this temperature for $2\frac{3}{4}$ hr.

A taste panel of 6 members evaluated the potatoes at each frying period. Samples were scored for appearance, odor, flavor, and texture on a 5-point scale. The top of the scale, 5, represented French fried potatoes of superior quality, whereas the bottom of the scale rated the potatoes unacceptable. Potatoes were fried in the fat cared for by each method until the panel scored them unacceptable.

The percentage of the original weight of fat used each frying period was calculated for each method of care. Also, the percentage decrease in weight of potatoes during frying was determined for 1 batch, selected at random, for each method of care.

After each frying period, the viscosity of the fat was determined in a MacMichael viscosimeter. The sample of fat was heated to 100° C and 3 readings were made for each sample. Acid numbers were determined, as described by Jacobs *(3)*, for samples of fat removed from each fryer at the completion of each frying period and after overnight storage. The samples were stored in sealed, glass jars with screw caps at −20° F (−28.9° C) until analyzed (approximately 12 wk).

The percentage of fat in the French fried potatoes was determined by a chemical and a physical method. At every frying period for each method of care, samples of potatoes (approximately 250 g) from the final batch were allowed to cool in a single layer on kraft paper towel. Each sample was wrapped in heavy aluminum foil using a drug store wrap, frozen, and stored at −20° F (−28.9° C) until analyzed (approximately 18 wk).

The chemical method was a modification of the method

[a] Contribution No 225, Department of Home Economics, Kansas Agricultural Experiment Station, Kansas State University, Manhattan.

[b] Presented at the Twentieth Annual Meeting of the IFT, San Francisco, California, May 16, 1960.

described by Jacobs *(3)* for determining the fat content of milk. Nine g of frozen potatoes were chopped finely and placed in a 100-ml beaker, 10 ml water added, and the mixture stirred with a glass rod. Glacial acetic acid (5 ml) was added, the mixture stirred to break up lumps, and finally sulfuric acid (10 ml) was added and the mixture stirred until the potatoes were dispersed completely. The remainder of the procedure followed that described by Jacobs *(3)*.

The Carver Laboratory Press was used for the physical method. The procedure was an adaptation of the method described by Weissberger *(6)*. The 1½-in cylinder of the press was lined with a pre-weighed 5-in square of cheese cloth of double thickness and a 25-cm circle of Whatman No 1 filter paper. Frozen, chopped potatoes (25 g) were packed in 3 layers alternating with 4 weighed 5.5-cm circles of filter paper. The ram was inserted after the cheese cloth was folded over the potatoes and filter paper. The plates of the Carver Press were preheated to 110° C; the packed cylinder was set on a drip pan, and placed on the lower plate. The lower plate was raised until contact was made between the top of the ram and the upper plate. The following schedule was used for applying pressure in pounds per square inch registered on the hydraulic gauge:

0–15 min, preheat
15–16 min, 250 psig
16–18 min, 500 psig
18–20 min, 750 psig
20–23 min, 1000 psig
23–25 min, 1250 psig
26–30 min, hold at 1250 psig

The percentage of fat expressed from the potatoes was calculated from the weight of the sample and the weight of the pressed briquette (the materials packed in the cylinder).

## RESULTS AND DISCUSSION

**Frying life and fat used per serving.** The method of care affected the number of times the fat could be used for frying potatoes. The frying life of the fat cared for by each method is shown by the number of periods listed in Table 1. Fat cared for by Method IV had a frying life of twenty-one 8-hr periods. Method III differed from Method IV in that the fat was filtered and the fryer cleaned with a commercial cleaner, rinsed, and dried. These conditions increased the frying life of the fat by 7 periods. When the fat turnover was 20% (Method II) instead of approximately 7% (Method III) and all other conditions were the same, 25 periods were added to the frying life. In Method 1 refrigerated storage was added to the conditions of Method II, and the frying life was increased 10 periods over the number for Method II. The greatest difference among methods of care occurred between the methods where the fat turnover was 20% (I and II) and those where it was 7% (III and IV), Table 1.

Although the frying life of fat cared for by Methods I and II was more than twice that of fat cared for by Methods III and IV, the quantity used to fry a 100 g serving of potatoes also was approximately twice that used by Methods III and IV (Table 1). This difference would have an important effect on the cost per serving of potatoes. For example, if the fat cost 30 cents per lb, the difference in raw food cost for frying potatoes in fat cared for by Methods I and II or by Methods III and IV would be approximately ½ cent per serving. However, a 100% fat turnover would be required more than twice as often for Methods III and IV, in which there was a 7% fat turnover after each 8 hr frying period, than for Methods I and II, in which there was a 20% fat turnover after each 8 hr period. A 100% fat turnover after every 21 to 28 frying periods may be more expensive than a 20% fat turnover after each 8 hr period. A 100% fat turnover after every 21 to 28 frying periods may be more expensive than a 20% fat turnover after each 8 hr frying period for 53 to 63 periods.

TABLE 1

**Frying periods and mean values for grams of fat used per 100 g potatoes, frying losses, and viscosity of the fat**

| Method | Periods | Fat to fry 100 g potatoes, g | Fat frying loss, % | Potato frying loss, % | Fat viscosity MacMichael |
|---|---|---|---|---|---|
| I | 63 | 16.1 | 7.3 | 52.0 * | 16.9 |
| II | 53 | 16.8 | 7.4 * | 50.0 | 16.8 * |
| III | 28 | 7.9 | 6.9 * | 50.2 * | 16.1 * |
| IV | 21 | 8.4 | 6.6 * | 47.0 * | 17.3 |

* Significant at the 5% level.

**Frying losses and viscosity of the fat.** Analyses of variance showed significant ($P<.001$) differences among the method of care for percentage weight loss of fat and potatoes during frying. Least significant differences among the means pointed out that the percentage of fat lost during frying was significantly ($P<.05$) greater for Methods I and II than for Methods III and IV. Differences between Methods I and II and between III and IV were nonsignificant (Table 1). These findings follow the same pattern as data for g of fat used to fry 100 g of potatoes.

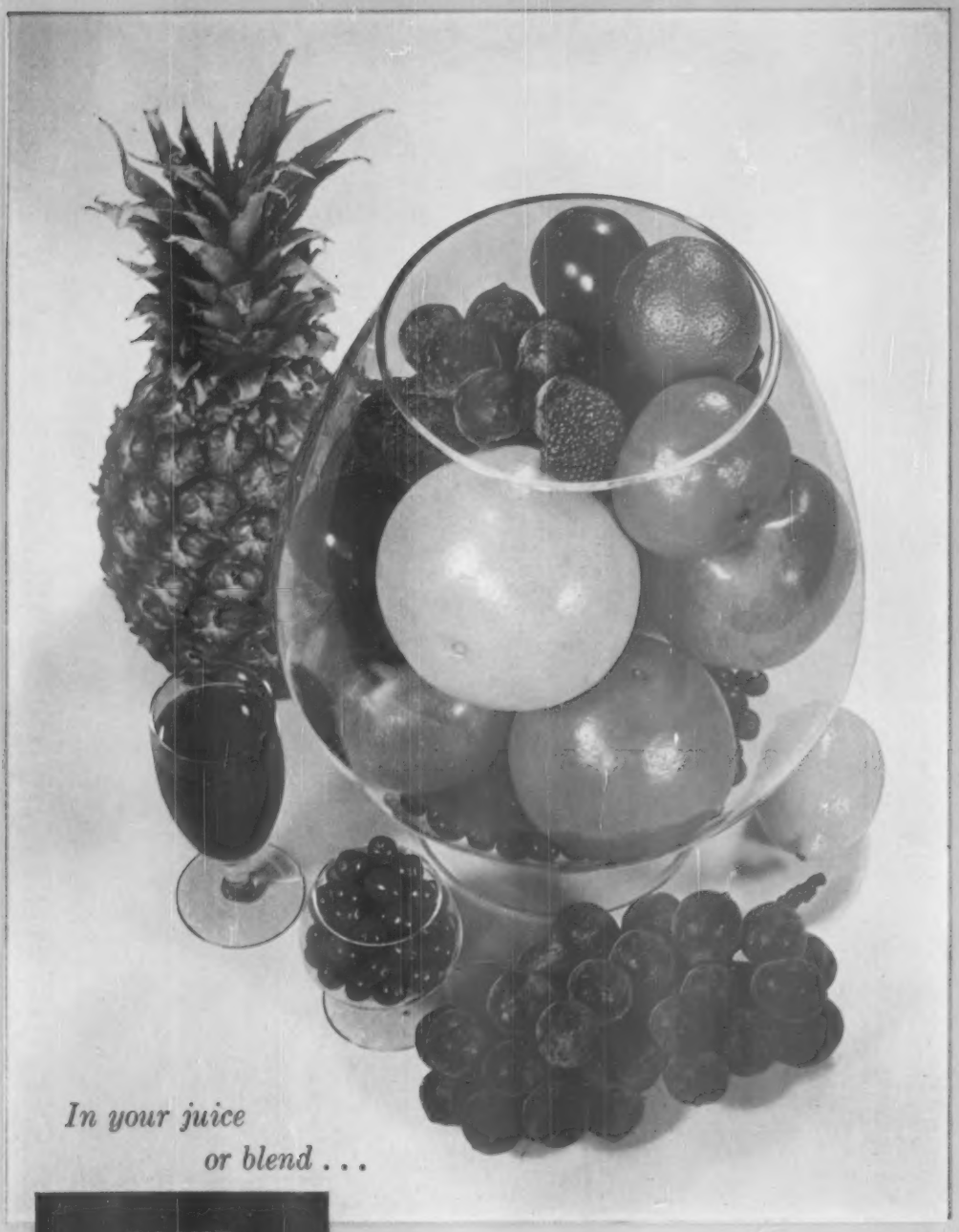
Regression coefficients indicated that for all of the methods fat loss increased significantly ($P<.001$) with additional frying periods. Values for t pointed out that the greatest increase in fat loss with additional frying occurred under Method IV and the least under Method II. Methods I and III were equal in this respect and intermediate between II and IV. Regression coefficients for each method were: I, +.041; II, +.023; III, +.050; and IV, +.062.

Least significant differences among the means for percentage weight loss of potatoes were calculated. Potatoes fried in fat cared for by Method I lost significantly ($P<.05$) more weight than potatoes fried in fat cared for by any of the other methods (Table 1). Also Method III contributed to significantly ($P<.05$) greater weight loss from potatoes than Method IV. Differences between Methods II and III were nonsignificant. Regression coefficients for each method showed no significant linear change in the weight loss of potatoes as the number of frying periods increased.

Differences in the viscosity of the fat, attributable to the method of care, were significant at the 5% level. MacMichael readings (Table 1) for fat at 100° C averaged significantly ($P<.05$) lower for fat cared for by Method III than those for fat under any other method. Differences between Methods I and II were nonsignificant.

There may be some relationship between the viscosity of the fat and weight loss of potatoes during

*Keep Faith with Nature—Guard her Goodness*



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frying. It is possible that the smaller the weight loss from the potatoes, the smaller the quantity of water transferred from the potatoes to the fat. The mean loss from potatoes fried in fat cared for by Method IV was lower than the mean losses from potatoes fried in fat cared for by any of the other methods. The mean viscosity measurement for fat cared for by Method IV was higher than mean measurements for fat handled by the other methods. This is an interesting trend even though differences between Method IV and other methods were not always significant.

**Palatability of potatoes and acid numbers for frying fat.** Analyses of variance were run on scores for appearance, odor, flavor, and texture of the potatoes and on acid numbers for samples of fat taken immediately after each frying period and after overnight storage. There were no significant differences in any of these factors that could be attributed to the method of care of the fat. This is to be expected, because the fat cared for by each method was used until palatability scores indicated that the potatoes were unacceptable.

The scores for each palatability factor rated the potatoes average (approximately 3) for the first few frying periods, then gradually increased to a superior rating (nearly 5) for most of the frying life of the fat. During the 3 final periods for each method of care the scores dropped to unacceptable (1 or less). The curves for flavor scores, given in Figure 1, illustrate the pattern for all palatability factors. Apparently the palatability panel preferred potatoes cooked in fat that had been used a short time to those cooked in fresh fat or in fat that had been used many times. The scores both increased, then decreased earlier in the series of frying periods for potatoes cooked in fat handled by Methods III and IV than by Methods 1 and II.

The acid numbers for fresh fat were zero. The rate of increase in acid numbers during frying varied with the method of care (Figure 2). The first free fatty acids appeared in the sample of fat cared for by Method IV at the second frying period. The acid number for the sample taken immediately after frying was 1.261. Samples of fat cared for by Methods II and III first had free fatty acids at the fourth period with acid numbers of 0.011 and 0.050, respectively. The first free fatty acid in fat handled by Method I was apparent at the fifth period with an acid number of 0.003. The acid numbers for fat treated by Methods I, II, and III gradually increased with each additional frying period up to the last 2 to 7 periods, then decreased slightly. Acid numbers for Method IV gradually increased with each additional period (Figure 2). Lowe *et al (4)* reported that the free fatty acid content of 20 fats increased during heating of the fats and cooking of doughnuts. The work of Bennion and Hanning *(1)* also agreed with this finding.

In the present study, acid numbers (mgKOH/g fat) for samples of fat taken immediately after the final frying period for each method were: I (n = 63), 23.243; II (n = 53), 23.001; III (n = 28), 23.802; and IV (n = 21), 23.049. In general, at all frying periods, acid numbers for fat stored overnight were slightly lower than those for the samples taken immediately after frying. However, acid numbers for fat handled by Method IV tended to increase slightly or remain about the same with overnight storage.

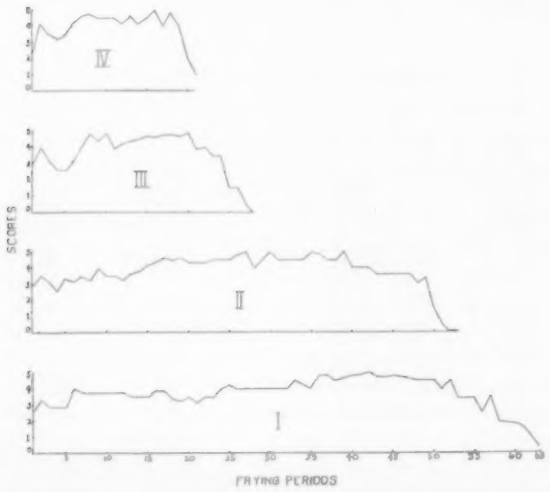


**Figure 1. Curves for flavor scores given potatoes fried in fat cared for by 4 methods.**

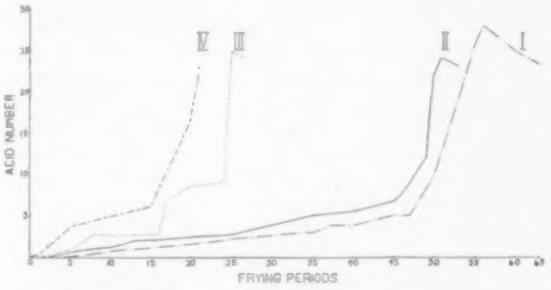


**Figure 2. Curves for acid numbers determined on samples of fat taken immediately after frying.**

Analysis of variance of acid numbers for the first 21 frying periods indicated significant ($P<.01$) variation among the methods of care. Least significant differences (Table 2) attributed this variation to significant ($P<.05$) differences between mean acid numbers for fat cared for by Methods I and II (20% fat turnover) and those for fat cared for by Methods III and IV (7% fat turnover). Also, least significant differences showed significant ($P<.05$) variation between mean acid numbers for samples of fat handled by Methods III and IV. Therefore, filtering the fat and cleaning, rinsing, and drying the fryer between frying periods reduced the average acid number for the first 21 periods.

Correlation coefficients for the scores for 3 palatability factors of potatoes and acid numbers for the fat are presented in Table 3. All of the coefficients were

TABLE 2

**Mean acid numbers for the first 21 frying periods**

| Method | Acid number, mg KOH/g fat | |
|---|---|---|
| | After frying | Over night |
| I | 0.878 | 0.246 |
| II | 1.497 | 1.079 |
| III | 3.527 * | 2.623 * |
| IV | 5.802 * | 6.249 * |
| lsd | 1.398 | 1.440 |

lsd = least significant difference at the 5% level.
* Significant at the 5% level.

negative and significant ($P<.01$ and .001). These indicated that, in general, as free fatty acids developed in the fat the palatability of the potatoes decreased. Moreover, Figures 1 and 2 show that for each method a sharp dip in flavor scores and a sharp increase in acid numbers occurred after approximately the same number of frying periods. Abrupt changes in flavor began after 52, 39, 20, and 18 periods; and sharp increases in acid numbers occurred after 47, 45, 16, and 15 periods for Methods I, II, III, and IV, respectively. Therefore, fat similar to that used in this study probably should not be used longer than 47 to 52 eight hr-frying periods when cared for by Methods I, 39 to 45 periods by Method II, 16 to 20 periods by Method III, and 15 to 18 periods by Method IV.

**Fat in cooked potatoes.** A chemical and a physical method were used to determine the percentage of fat in the cooked potatoes. Analysis of variance showed that the method of caring for the fat had no significant effect on the percentage of fat recovered from the potatoes as determined by either method. Mean values and the range in values for the percentage of fat in the potatoes are given in Table 4. On the average, approximately 3 to 4% less fat was found by the chemical method than by the physical method. Correlation coefficients for the chemical and physical measurements (Table 4) were nonsignificant except when the fat was cared for by Method III. The correlation coefficient for this method is rather weak, because it leaves 5/6 of the variance under one measurement unassociated with variance under the other measurement.

TABLE 3

**Correlation coefficients for palatability scores for potatoes and acid numbers for fat**

| Factor | Method | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| | n = 63 | n = 53 | n = 28 | n = 21 |
| Odor | | | | |
| After frying | −.720*** | −.652*** | −.810*** | −.880*** |
| Overnight | −.731*** | −.641*** | −.828*** | −.885*** |
| Flavor | | | | |
| After frying | −.494*** | −.679*** | −.621*** | −.603** |
| Overnight | −.520*** | −.700*** | −.649*** | −.604** |
| Texture | | | | |
| After frying | −.628*** | −.519*** | −.590*** | −.577** |
| Overnight | −.639*** | −.510*** | −.610*** | −.570** |

** Significant at the 1% level.
*** Significant at the 0.1% level.

**Observations on the frying fat and potatoes.** Samples of fat collected during the experiment were arranged according to the method of care for the fat and in chronological order by frying periods. Samples representing all methods of care became darker as the frying periods increased. Samples taken from the final period for each method of care were all about the same dark brown color. At room temperature each of these 4 samples had a pronounced acrid odor. Bennion and Hanning *(1)* reported similar results when fritter batter was fried in continuous process lard.

The potatoes fried during the last two periods for each method of care did not brown. These potatoes had the appearance of raw potatoes dipped in fat. However, the palatability panel commented that the potatoes were cooked, but limp and soaked with fat.

TABLE 4

**Percentage of fat in fried potatoes as determined by chemical and physical measurements and correlation coefficients for the 2 measurements**

| Method | Chemical measurement | Physical measurement | Correlation coefficient |
|---|---|---|---|
| I n = 63 | | | |
| Mean | 14.7 | 17.7 | |
| Range | 12.3–18.0 | 15.2–23.1 | |
| r | | | −.154 |
| II n = 53 | | | |
| Mean | 13.7 | 17.4 | |
| Range | 12.0–16.6 | 15.8–19.1 | |
| r | | | −.025 |
| III n = 28 | | | |
| Mean | 14.7 | 17.5 | |
| Range | 12.7–15.9 | 16.0–18.9 | |
| r | | | +.429* |
| IV n = 21 | | | |
| Mean | 14.3 | 17.7 | |
| Range | 12.1–16.5 | 16.7–18.5 | |
| r | | | +.129 |
| Pooled n = 165 | | | |
| Mean | 14.4 | 17.6 | |
| Range | 12.0–18.0 | 15.2–23.1 | |
| r | | | +.156* |

* Significant at the 5% level.

## SUMMARY AND CONCLUSIONS

The effect of 4 methods of care on the frying life of a hydrogenated vegetable fat processed for food service deep fat frying was investigated. The methods of care were: I—filtered, stored in a covered metal container at 2° to 5° C, and 20% (of original weight) fresh fat added each 8-hr frying period; II—filtered, stored in a cleaned fryer at 20° to 25° C, and 20% fresh fat added each frying period; III—filtered, stored in a cleaned fryer at 20° to 25° C and fresh fat added to replace that lost during each 8-hr frying period; IV—skimmed, stored in fryer at 20° to 25° C, and fresh fat added to replace that lost at each frying period.

Frying life of the fat was determined by palatability scores for French fried potatoes. Fat cared for by Methods I and II had a frying life of more than

twice that of fat caried for by Methods III and IV. Methods I and II also required approximately twice as much fat as Methods III and IV to fry 100 g of potatoes, which would affect the cost of a serving of French fried potatoes. The cost of a 100% fat turnover that would be required more than twice as often for Methods III and IV than for Methods I and II should be considered. Filtering and refrigerating the fat and cleaning the fryer between frying periods also prolonged the frying life of the fat.

Regardless of method of care, there was a significant negative correlation between the acid number of the fat and the acceptability of French fried potatoes. The method of care significantly affected the viscosity of the fat and the percentage weight loss of fat and potatoes during frying. In general, there was little correlation between values for a chemical and a physical method used to determine the percentage of fat in the fried potatoes.

**Acknowledgment**

Appreciation is expressed to Dr. H. C. Fryer, Head of the Department of Statistics, for advice in designing the experiment and analyses of the data.

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# The Effectiveness of Acronize Chlortetracycline in Poultry Preservation Following Long Term Commercial Use*

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(Manuscript received May 17, 1960)

On november 30, 1955, the Federal Food and Drug Administration cleared the use of the antibiotic chlortetracycline (CTC) for extending the shelf-life of poultry by stating, "A tolerance of 7 ppm is established for residues of chlortetracycline in and on uncooked poultry and this tolerance level should not be exceeded in any part of the poultry." Since then, Acronize chlortetracycline has been used effectively by the poultry processing industry to retard bacterial spoilage and thus maintain freshness. Many workers in the past 4 years have demonstrated the effectiveness of Acronize on poultry *(3, 4, 7, 8, 9, 10, 12, 13, 14, 16)*. It would now seem appropriate to evaluate the current effectiveness of the antibiotic after continued plant use and compare it with its original ability to retard spoilage. As a result of extensive field experience, it is postulated that there is no decrease in the effectiveness of Acronize and no build-up of insensitive microorganisms in poultry processing plants after long term commercial use. The present experiments were undertaken to test this postulate.

## EXPERIMENTAL PROCEDURE

**1. Microbial counts.** Microbial counts were determined on poultry essentially by a modification of the method described by Kohler *et al (7, 8)*.

**2. Chlortetracycline tissue residues.** In Trials 1 and 2 (1954 and 1957, respectively), CTC residues in poultry tissues were determined as described by Abbey *et al (1)*. A modified cylinder plate method *(6)* was employed for all tissues bioassayed in Trial 3. One part of tissue (5 g) was Waring blended with 4 parts (20 ml) of pH 4.5 potassium phosphate buffer and allowed to settle. The supernatant was used for the determination.

* Presented at the Twentieth Annual Meeting of the Institute of Food Technologists, San Francisco, California, May 17, 1960.

**3. Organoleptic evaluation.** The odor evaluation technique was employed by a tested and trained panel consisting of at least 5–6 laboratory personnel *(5)*. The mean average grade of each group was plotted on linear graph paper (rating *vs* time). The spoilage time and end of shelf life was estimated where these lines cross 5.5. An average score of 5.5 indicates that the birds would no longer be acceptable to the consumer as judged by our test panels. The grading system used was as follows:

Acceptable

10—Fresh odor (excellent)
9—
8—Very slight off-odor
7—
6—Slight off-odor
5.5

Not Acceptable

5—
4—Medium off-odor (unpleasant)
3—
2—Strong off-odor (offensive)
1—Very strong off-odor (putrid)

## RESULTS

All trials were conducted at a nearby poultry processing plant under commercial conditions. The samples were returned to the laboratories for storage under various refrigerated conditions. All procedures are similar unless specified otherwise in the individual trials.

*Trial 1—January, 1954.* Freshly killed, whole, eviscerated chickens were held for 2 hr in a chill tank containing ice and water plus 20 ppm chlortetracycline as Aeronize PD. All birds were drained and either packed in ice, or dry-packed and shipped in sealed cartons to a poultry wholesaler where they were stored under commercial conditions (3° C) for the duration of the experiment. Controls were processed in a similar manner.

After 3 weeks storage the Acronized* poultry was in excellent condition while the respective control group was putrid. Table 1 shows the data and odor scores relative to this trial. Birds processed with Acronize showed a 38% increase in shelf-life when shipped in ice, while those shipped dry-packed showed a further increase to 73% shelf-life.

This initial commercial trial clearly demonstrated that the use of 20 ppm chlortetracycline in the cooling water, as used in commercial poultry processing, markedly increased the shelf-life of poultry. These commercial results correlated well with previous laboratory studies. Kohler *et al (8)* reported this study in an earlier paper.

*Trial 2—March, 1957.* Freshly killed, whole, eviscerated broilers were processed with 20 ppm chlortetracycline as Acronize PD for 2–3 hr in chill tanks. After draining, the birds were bagged in polyethylene, crated in ice and returned to the laboratories. They were then stored at 3° C for the duration of the experiment. Control poultry was prepared in a similar manner. The usual microbiological and organoleptic observations were made throughout the storage period. In addition, microbial counts were also done with nutrient agar containing 20 ppm chlortetracycline to estimate the number of insensitive organisms. The test results are outlined in Tables 2, 3, and 4.

In this trial an 84% increase in shelf-life was found for Acronized birds over the untreated controls. The plate counts done with added CTC indicated a marked decrease in total bacterial population. A greater percentage of insensitive organisms grew out from the Acronized poultry than from the untreated controls as shown in Table 3.

*Trial 3—December, 1959.* A repeat of Trial 2 was fulfilled at the same poultry plant. Again, freshly killed, whole, eviscerated broilers were processed commercially with and without Acronize for 2 hr in chill tanks. The birds were drained and some were placed in individual polyethylene bags while the remainder were held naked, in ice. All samples were iced and returned to the laboratories where they were stored at 3° C or in ice until spoiled. At specific time intervals, replicate samples were removed from each group for microbial and organoleptic evaluation; iced birds were assayed for CTC at the same sampling times. Tables 5, 6, and 7 show the spoilage pattern, insensitive organisms and CTC assays respectively.

Acronized poultry stored in ice showed 57% increase in shelf life but when stored in polyethylene bags, an increase to

* Trademark.

**TABLE 1**

**Trial 1 (1954)—Preservation of poultry and organoleptic scores under commercial conditions**

| Storage conditions | Treatment | Av microorganisms x $10^6$/gram (3 birds/group) Days in cold storage (3° C) | | | | | Spoilage day |
|---|---|---|---|---|---|---|---|
| | | 0 | 7 | 14 | 21 | 28 | |
| Dry packed | Control | 0.0016(10) | 0.138(10) | 1390(6) | 5400(1) | 3500(1) | 14.8 |
| | Acronize | ..... | 0.004(10) | 0.77(10) | 10.7(10) | 134(2) | 25 |
| Iced | Control | 0.0016(10) | 0.068(10) | 175(8) | 1830(1) | 4150(1) | 17.8 |
| | Acronize | ..... | 0.365(10) | 0.35(10) | 15(8) | 255(2) | 24.5 |

( ) = Organoleptic Scores.
10 = Fresh odor (excellent). 8 = Very slight off-odor. 6 = Slight off-odor. 4 = Medium off-odor. 2 = Strong off-odor. 1 = Putrid.

**TABLE 2**

**Trial 2 (1957)—Preservation of poultry with chlortetracycline and organoleptic scores under commercial conditions**

| Treatment | Av microorganisms x $10^6$/gram (5 birds/group) Days in cold storage (3° C) | | | | | | | Spoilage day |
|---|---|---|---|---|---|---|---|---|
| | 1 | 8 | 12 | 14 | 16 | 19 | 22 | |
| Control | 0.0073(10) | 1.45(10) | 61(6) | 57(4.8) | 111(4) | 248(3.1) | 138(2.7) | 12.8 |
| Acronize | 0.0039(10) | 0.19(10) | 1.7(9) | 8.6(8.5) | 21(8) | 133(6) | 276(6) | 23.6 |

( ) = Organoleptic Scores.

**TABLE 3**

**Trial 2 (1957)—Insensitive bacterial population and percentage of total population inhibited by chlortetracycline under commercial conditions**

| Treatment | Av microorganisms x $10^6$/gram (5 birds/group) Days in cold storage (3° C) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | (%) | 8 | (%) | 12 | (%) | 14 | (%) | 16 | (%) | 19 | (%) | 22 | (%) |
| Control | 0.00018 | (75) | 0.022 | (98.5) | 4.06 | (93.5) | 4.2 | (93) | 10 | (91) | 45 | (82) | 35 | (75) |
| Acronize | 0.00035 | (89) | 0.078 | (59) | 0.55 | (69) | 2.8 | (67.5) | 3.7 | (82.5) | 62 | (53.5) | 164 | (41) |

(%) = Per cent of total population inhibited by chlortetracycline in agar.

TABLE 4

Trial 2 (1957)—Chlortetracycline distribution in poultry treated under commercial conditions

| Treatment | Av mcg chlortetracycline/gram tissue (5 birds/group) | | | | | |
|---|---|---|---|---|---|---|
| | Breast skin | Top breast | Deep breast | Deep thigh | Shoulder | Back skin |
| Control | Neg | Neg | Neg | Neg | Neg | Neg |
| Acronize | 1.94 | 0.12 | Neg–0.04 | 0.12 | 0.26 | 2.57 |

80% over the control was noted. This compared favorably with the earlier results in Trial 1. The data in Table 6 again indicated a marked reduction in total bacterial counts when 20 ppm chlortetracycline was added to the plating agar. However, a greater percentage of insensitive organisms was found on the antibiotic-treated poultry when compared with the untreated controls. The initial tissue levels of CTC were comparable to those found in other trials, but the amount of activity tended to decrease with increased storage time in ice. This is especially true of the outer surfaces while the deeper tissues remained relatively constant.

## DISCUSSION

It has been established that chlortetracycline is the most effective antibiotic for poultry preservation *(2, 8, 10, 13, 16, 19)*. In January, 1954, when Trial 1 was conducted, Acronize effectively prolonged shelf-life by 73%; in March, 1957, (Trial 2), by 84%; and in December 1959, (Trial 3), by 80%. On this basis, the efficacy of Acronize appears to be the same over a period of 5 years of repeated use. In the two trials (1 and 3) where poultry was stored naked in ice, it was noted that the Acronize shelf-life extension (38% and 57%, respectively, over the control) tended to be less than that stored in polyethylene bags. Although the temperature of the poultry was held closer to 0° C under these conditions, the continuous leaching by melting ice tended to reduce the amount of available antibiotic. This may have in turn decreased the bacteriostatic level (approximately 1 ppm) on the skin of the bird and spoilage then might take place more readily. This was confirmed in Table 7 where the initial skin levels (2.6 and 2.8 ppm) dropped to about 0.5 over a 3-week storage period in ice. Control poultry in Tables 1 and 5 did remain acceptable for a longer period of time in ice because of their lower storage temperature.

In the past several years, it has been suggested that the widespread and continued use of Acronize could

TABLE 5

Trial 3 (1959)—Preservation of poultry and organoleptic scores under commercial conditions

| Treatment | Av microorganisms x $10^6$/gram (5 birds/group) Days in cold storage (3° C) | | | | | | | Spoilage day |
|---|---|---|---|---|---|---|---|---|
| | 0 | 3 | 7 | 10 | 14 | 17 | 21 | |
| Control (bagged) | 0.032(10) | 0.052(9.3) | 8.82(7.6) | 217(5.7) | 584(3.3) | 730(3.0) | 672(1.2) | 10.3 |
| Control (iced) | 0.032(10) | 0.050(9.7) | 1.55(8.8) | 71(6.3) | 116(4.0) | 738(3.0) | 960(1.8) | 11.3 |
| Acronize (bagged) | 0.025(10) | 0.012(9.5) | 0.011(9.2) | 0.023(8.0) | 6.5(6.8) | 56(6.4) | 236(4.2) | 18.6 |
| Acronize (iced) | 0.025(10) | 0.011(9.7) | 0.035(9.8) | 0.51(8.7) | 29.3(6.8) | 106(6.0) | 539(3.4) | 17.7 |

( ) = Organoleptic Scores.

TABLE 6

Trial 3 (1959)—Insensitive bacterial population and percentage of total population inhibited by chlortetracycline under commercial conditions

| Treatment | Av microorganisms x $10^6$/gram (5 birds/group) Days in cold storage (3° C) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | (%) | 3 | (%) | 7 | (%) | 10 | (%) | 14 | (%) | 17 | (%) | 21 | (%) |
| Control (bagged) | 0.004 | (87.5) | 0.002 | (97.4) | 0.090 | (99) | 6.85 | (97) | 29.7 | (94.9) | 33 | (95.5) | 16.7 | (97.5) |
| Control (iced) | 0.004 | (87.5) | 0.001 | (98) | 0.026 | (98.3) | 1.92 | (96.5) | 3.8 | (96.5) | 29 | (96) | 18.1 | (98.1) |
| Acronize (bagged) | 0.003 | (88) | 0.002 | (83.3) | 0.003 | (73) | 0.08 | (65) | 1.1 | (83) | 13.6 | (76) | 64 | (73) |
| Acronize (iced) | 0.005 | (80) | 0.002 | (81.8) | 0.004 | (89) | 0.15 | (65) | 9.2 | (62.5) | 26.5 | (75) | 114 | (79) |

(%) = Per cent of total population inhibited by chlortetracycline in agar.

TABLE 7

Trial 3 (1959)—Chlortetracycline (CTC) distribution in poultry stored in ice for three weeks

| Treatment | Days in ice storage after treatment | Av mcg chlortetracycline/gram tissue (5 birds/group) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Breast skin | Top breast | Deep breast | Thigh | Shoulder | Back skin |
| Control | 0 | Neg–0.02 | Neg–0.02 | Neg | Neg–0.03 | Neg | Neg |
| | 3 | Neg–0.04 | Neg | Neg | Neg | Neg–0.02 | 0.02 |
| | 7 | Neg–0.03 | Neg | Neg | Neg | Neg | Neg |
| | 10 | Neg | Neg | Neg | Neg | Neg | Neg |
| | 14 | Neg | Neg | Neg | Neg | Neg | Neg |
| | 17 | Neg | Neg–0.02 | Neg | Neg–0.02 | Neg | Neg |
| | 21 | Neg | Neg | Neg | Neg | Neg | Neg |
| Acronize | 0 | 2.6 | 0.39 | 0.11 | 0.32 | 0.20 | 2.81 |
| | 3 | 1.74 | 0.63 | 0.12 | 0.63 | 0.60 | 2.38 |
| | 7 | 1.03 | 0.50 | Neg–0.06 | 0.62 | 0.98 | 1.28 |
| | 10 | 0.59 | 0.30 | 0.04 | 0.67 | 0.56 | 1.09 |
| | 14 | 0.67 | 0.32 | 0.06 | 0.21 | 0.54 | 0.73 |
| | 17 | 0.42 | 0.33 | 0.08 | 0.30 | 0.78 | 0.67 |
| | 21 | 0.34 | 0.37 | 0.11 | 0.24 | 0.41 | 1.00 |

cause an increase of "insensitive" organisms *(11, 15, 18)*. It was implied that few, if any, of a naturally occurring population were resistant prior to the advent of Aeronize chlortetracycline and that, with the continued use of the antibiotic, resistance was developed in formerly sensitive organisms *(11)*. Our data suggest the contrary. In Table 3 (Trial 2—March, 1957), 75% of the initial total population on control poultry was inhibited by 20 ppm chlortetracycline in the plating agar whereas 89% of the initial total population of the Acronized poultry was inhibited. Almost 3 years later, in the same poultry processing plant and after continued use of Aeronize (Table 6, Trial 3—December, 1959), the data indicate that 87.5% and 88% of the initial total population were inhibited by 20 ppm chlortetracycline in the plating agar for control and CTC groups, respectively. This suggested to us that there was no "development" of insensitive organisms but that the antibiotic acted in a selective manner to control the spoilage microorganisms. It would seem that a certain percentage of the organisms in the normal bacterial population is naturally insensitive to the antibiotic, and when the others are controlled, these insensitive ones may become a larger percentage of the remaining population, at the time of spoilage. This may be the case, since Acronize has not lost its effectiveness for poultry preservation in the past 5 years. Our beliefs were recently confirmed by Vaughn *et al (17)* who observed that " . . . microorganisms acquire resistance to CTC in a stepwise manner and that highly sensitive organisms are difficult to train." They also stated, "It is unlikely that resistant organisms in a poultry processing plant result from training of the resident nonresistant population. A more likely explanation is that there is an active selection for the small number of resistant organisms already present and that conditions in the plant are such that multiplication can occur."

## SUMMARY

Acronize remained effective (75% average shelf-life extension) in the retardation of poultry spoilage after 5 years of continued commercial use.

Acronized poultry held under ice demonstrated an increase (48% average) in shelf-life over control poultry. When poultry was stored in polyethylene bags, an increase (77% average) in shelf-life over the control is noted.

The indications are that an increase of insensitive microorganisms in a developing spoilage population on Acronized poultry is most likely caused by "selection" rather than "development."

### Acknowledgment

Grateful appreciation is made to Mr. Herman Berenson and Mr. David Hewel for their technical assistance and participation in the field trials and the bacteriological work. We are especially thankful to the Producers Cooperative Exchange, Coatesville, Pennsylvania, for the use of certain facilities in their poultry processing plant.

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# The Effect of Number of Judgments in a Test on Flavor Evaluations for Preference[a, b]

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(Manuscript received May 18, 1960)

THE NUMBER of samples which may be served to a flavor panel at one time is important from both an economical and efficiency standpoint. Some research *(5, 7, 9, 10)* has indicated that discrimination or the ability to detect flavor differences decreases with an increasing number of samples. This is generally attributed to either psychological fatigue of the judge *(4)* or to desensitizing of the taste buds *(2)*. Other research *(3, 8)* has indicated that as many as 15 samples may be served at one session without encountering loss of sensitivity. As there is lack of agreement between laboratories as to the maximum number of samples which may be served in one test, this study was designed to evaluate the discrimination ability of the judge when 20 different samples of the same product are included in one test session.

## MATERIALS AND METHODS

Four products were selected for testing on the basis of securing different types of food products. The products selected were canned peaches, canned green beans, broiled hamburger patties, and tomato juice.

Twenty different varieties of peaches were grown on the College Horticultural Farm and processed in the Food Technology pilot plant following recommended commercial procedures. The sliced peaches were canned in August and stored at room temperature until tested on October 31, 1958.

On November 4, 160 lb of hamburger containing approximately 20% fat was secured from a U. S. Good grade beef carcass. The hamburger was divided into 8-lb lots and different seasonings added to each of the 20 lots. The hamburger was held at 34° F overnight and tested on November 5.

Sixty gallons of a single commercial brand of tomato juice was purchased. The tomato juice was mixed together in one large tank and then divided into twenty 3-gal lots. Different seasonings were added to each of the 20 different lots.

FM-1 Blue Lake variety of green beans was secured from the College Horticultural Farm and processed in the Food Technology pilot plant following recommended commercial procedures. Different seasonings were weighed into each can just prior to sealing and retorting. Twenty-four No 303 cans of each of 20 different seasoning variations were canned.

For serving to the panel, each pound of hamburger was made into 6 patties. The patties were broiled in a gas broiler to an internal temperature of 170° F and ¼ patty served to each judge. The green beans were heated to approximately 180° F for serving and the tomato juice and peaches were served at room temperature.

When served to the judges, the 20 samples of each product were assigned to 4 trays. The same 5 samples were presented in random order and always served together on the same tray. The sample variations included on each tray are given in Tables 1, 2, 3, and 4. The trays were served to each judge sequentially with no time interval between trays. Each judge was allowed to take as much time as needed to test the 20 samples,—usually about 10 min. The order of the 4 trays of each product was assigned to the judges so as to have each tray tested in each possible order an equal number of times. For each product, each tray was judged in a given order by approximately 45 judges.

College students served as members of the flavor preference panel. They were not trained or pre-selected. Following a general notice to students during college registration, the first 200 students to apply were hired. The students were paid a small fee (35¢ per test period). Free coffee, punch and "treats", such as candy, gum, or cookies were available to them after testing. There were approximately twice as many boys as girls on the panel.

TABLE 1
Canned peaches

| Tray | Code number | Varieties |
|---|---|---|
| A | 415 | Elbertas |
| | 257 | Horstings |
| | 189 | Golden Hale |
| | 615 | Triogem |
| | 802 | Vee Freeze |
| B | 569 | Sungold |
| | 252 | Clyde deFollett |
| | 866 | FV 9-93 |
| | 504 | Wynsum |
| | 643 | Giant Elbertas |
| C | 606 | Fay Elbertas |
| | 034 | Viceroy |
| | 954 | Sunbeam |
| | 168 | Sunhigh |
| | 654 | Redskins |
| D | 564 | The Dalles No 5 |
| | 872 | July Elbertas |
| | 402 | Fairhaven |
| | 997 | Eclipse |
| | 234 | Merrill 49'ers |

TABLE 2
Hamburger

| Tray | Code number | Amount of seasoning added to each 8-lb lot treatments |
|---|---|---|
| A | 091 | 12 g Charcosalt, 14 ml liquid smoke |
| | 696 | 4 g onion powder |
| | 629 | 14 g B-B-Q spice |
| | 435 | 20 g Barbmust, 6 g salt |
| | 218 | 60 g seasoned salt |
| B | 774 | 30 g salt |
| | 680 | 16 g black pepper |
| | 784 | 16 g seasoned salt |
| | 966 | 14 ml liquid smoke, 6 g salt |
| | 079 | 60 g Barbmust |
| C | 017 | 60 g salt |
| | 437 | 8 g celery salt |
| | 805 | 10 ml liquid smoke, 6 g salt |
| | 113 | 12 g Charcosalt, 6 g garlic powder, 6 g celery salt |
| | 535 | Unseasoned |
| D | 236 | 30 g salt, 12 g black pepper |
| | 404 | 12 g garlic salt |
| | 171 | 18 g Charcosalt |
| | 294 | 40 g B-B-Q spice |
| | 421 | 15 ml liquid smoke, 15 ml spray smoke, 4 g onion powder, 20 g Barbmust |

[a] Technical Paper No. 1326, Oregon Agricultural Experiment Station. Contribution of the Departments of Food and Dairy Technology and Statistics.

[b] Presented at the Twentieth Annual Meeting of the Institute of Food Technologists, May 18, 1960, San Francisco, California.

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The judges were allowed to test at any time from 8:30 to 11:30 A.M. and 1:30 to 4:30 P.M. The samples were served in small paper cups, coded with three-digit random numbers, to the judges who were seated in individual flavor testing booths. The booths were uniformly lighted with white incandescent light and equipped with individual porcelain sinks, hot and cold water supply, paper drinking cups, pencils and service counter.

The judges were asked to score the samples on the following 9-point hedonic scale:

(9) Like Extremely
(8) Like Very Much
(7) Like Moderately
(6) Like Slightly
(5) Neither Like nor Dislike
(4) Dislike Slightly
(3) Dislike Moderately
(2) Dislike Very Much
(1) Dislike Extremely

The score points given in parentheses did not appear on the ballot but were assigned to the terms for the purpose of statistical analysis.

TABLE 3
Tomato juice

| Tray | Code number | Amount of seasoning added to each 3-gal lot treatments |
|---|---|---|
| A | 811 | No addition |
| | 276 | 40 ml 50% citric acid solution |
| | 492 | 60 g sugar |
| | 363 | 30 ml liquid smoke |
| | 292 | 8 g black pepper |
| B | 158 | 12 g salt |
| | 335 | 120 g sugar |
| | 508 | 15 ml garlic juice |
| | 323 | 5 g celery salt, 40 ml onion juice |
| | 752 | 30 ml liquid smoke, 15 g seasoned salt, 30 ml garlic juice |
| C | 425 | 24 g salt |
| | 447 | 10 ml onion juice |
| | 990 | 15 g seasoned salt |
| | 628 | 20 g celery salt |
| | 017 | 120 g sugar, 40 ml 50% citric acid solution |
| D | 358 | 20 ml 50% citric acid solution |
| | 214 | 10 g celery salt |
| | 824 | 30 ml garlic juice |
| | 232 | 8 g black pepper, 12 g salt |
| | 111 | 60 g sugar, 18 g salt |

TABLE 4
Green beans

| Tray | Code number | Amount of seasoning added to each No 303 can treatments |
|---|---|---|
| A | 124 | 7 g B-B-Q spice, 1.3 g salt |
| | 950 | 5.2 g salt |
| | 237 | 2.6 g salt, 1 g onion powder |
| | 663 | 2.6 g salt, 1 g celery salt |
| | 809 | 3.2 g salt, 1 g black pepper |
| B | 244 | 1.3 g salt |
| | 043 | 5 g Charcosalt |
| | 816 | 2.6 g salt, 2 g onion powder |
| | 164 | 2.6 g salt, 2 g celery salt |
| | 384 | 2.6 g salt, 1 g garlic powder |
| C | 640 | 2.6 g salt |
| | 723 | 1.3 g salt, 14 ml liquid smoke |
| | 594 | 1.3 g salt, 12 g ham seasoning |
| | 063 | 2.6 g salt, 5 g celery salt |
| | 938 | 2.6 g salt, 3 g garlic powder |
| D | 136 | 3.9 g salt |
| | 274 | 8 g Charcosalt |
| | 413 | 3.2 g salt, 0.5 g black pepper |
| | 636 | 1.3 g salt, 6 g ham seasoning |
| | 026 | Unseasoned |

## RESULTS AND DISCUSSION

Analyses of variance were run for each tray and order on each product and the treatment mean square against the judge by treatment interaction to obtain an F ratio representing ability of the test to discriminate differences among the samples. The F values are given in Table 5.

TABLE 5
F values

| Product | Order | Tray A | Tray B | Tray C | Tray D | Order means |
|---|---|---|---|---|---|---|
| Canned peaches | 1 | 4.06 | 13.42 | 17.38 | 4.93 | 9.96 |
| | 2 | 4.20 | 12.83 | 25.71 | 5.28 | 12.00 |
| | 3 | 6.49 | 16.63 | 25.95 | 7.84 | 14.23 |
| | 4 | 8.72 | 14.63 | 27.39 | 6.08 | 14.21 |
| Hamburger | 1 | 16.05 | 8.26 | 17.66 | 22.68 | 16.16 |
| | 2 | 30.41 | 22.09 | 9.08 | 32.57 | 26.04 |
| | 3 | 17.15 | 17.95 | 11.35 | 31.01 | 19.36 |
| | 4 | 30.62 | 10.88 | 16.04 | 33.87 | 22.85 |
| Tomato juice | 1 | 25.81 | 41.65 | 9.70 | 19.14 | 24.07 [1] |
| | 2 | 67.58 | 42.65 | 13.70 | 30.96 | 38.72 |
| | 3 | 44.22 | 66.45 | 11.90 | 23.41 | 36.47 |
| | 4 | 43.43 | 65.71 | 16.21 | 36.97 | 40.58 |
| Green beans | 1 | 43.04 | 5.10 | 21.64 | 27.81 | 24.39 |
| | 2 | 47.71 | 2.99 | 17.00 | 31.27 | 24.74 |
| | 3 | 49.46 | 4.00 | 19.48 | 31.63 | 26.14 |
| | 4 | 45.82 | 3.36 | 11.81 | 32.88 | 24.57 |

[1] Significantly different from means of orders 2, 3 and 4 at the .05 level.

When the F values are lower for a given tray, it indicates that there were smaller differences in flavor between the samples on this tray. In comparing the effect of order of serving, the higher F values indicate more ability to discriminate flavor preference differences between samples.

To test differences in discrimination due to order, an analysis of variance was run for each product on the square root of the F values. The transformation was made to help meet the assumptions of additivity and common variance *(1)*. Although there was some indication that the judges were able to discriminate less well on the first tray, as evidenced by the lower F values for three of the products, only on tomato juice did the first tray have a significantly lower F value.

The results can generally be said to corroborate previous reports that a warm-up session is of value *(6)* and at least for such mild products as included in this test, to make questionable reports which indicate that more than 5 to 8 samples at one sitting will markedly decrease ability to discriminate flavor differences.

## SUMMARY

For each of four products, canned peaches, hamburger, tomato juice, and green beans, 20 samples having known flavor differences were scored for preference by a panel of 175 to 191 untrained flavor judges. The 20 samples of each product were assigned to four trays and judged in one continuous test period. The order of presentation of the four trays to the judges was assigned so as to have each tray tested in each possible order an equal number of

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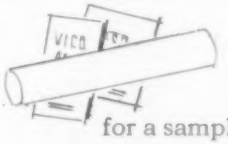

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times. It was assumed that the preference differences for the samples on a tray were flavor differences.

The results on tomato juice indicated the judges had a lower ability to detect differences among samples on the first tray than when the same samples were served on succeeding trays. On the other three products tested, (green beans, hamburger, and peaches), tray order did not significantly affect the ability of the judges to discriminate between samples. This indicates that for mild products such as included in this test, up to 20 samples may be included in one test period with no decrease in the judges' ability to discriminate flavor preferences among samples.

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# Studies on English (Persian) Walnuts, *Juglans regia*. II.

## Dehydration of Kernels with the Belt-Trough Dryer

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At ambient temperature, the stability of shelled walnuts is reduced when moisture levels deviate significantly from an optimum of 3.5 ± 0.3% *(7, 8, 11)*. Abrasion of the kernel and flaking of the pellicle accelerate the development of oxidative rancidity *(6)*. This is due to the increased exposed surface oil that exudes from ruptured cells and to the mechanical removal of pellicle which contains natural antioxidants *(3, 4)*.

The Romberg recovery process *(9, 10)* for the separation of walnut shells and kernels involves their rehydration in water and separation in brine solution. During this process, the recovered walnut meats attain moisture levels of 15% or more and some of the natural antioxidants may be lost by flaking or extraction of both the pellicle and kernel. It is advantageous that this process removes surface oil on abraded or fragmented kernels. However, dehydration to within the optimum moisture range must be affected within a short time in order to minimize kernel deterioration.

The cascade type dryer employed presently for drying high moisture kernels obtained from the recovery process affords minimum abrasion of kernels. However, a drying time of 100 to 120 minutes per unit batch is required for drying although ten successive batches are accommodated at one time. Other disadvantages of the cascade dryer include its large space requirement and the extensive periodic overhauls that are required to maintain optimum performance.

The belt-trough dryer *(5)* has the following advantages: it is highly efficient; it has a large capacity;

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it requires minimum space and maintenance; it operates continuously; and it effects rapid drying with minimum abrasion of soft and delicate food products *(1)*. Although the belt-trough dryer is normally employed for continuous operations, batch drying may be accomplished by bringing the belt to a horizontal position and closing the discharge port. The "batch" drying experiments described in the present paper were designed to determine (a) whether mixed sizes of fragmented kernels could be dried with minimum abrasion and without clogging or loss of fines through the wire belt and (b) optimum dehydration times and temperatures commensurate with the preservation of kernel quality.

A maximum drying temperature of 110° F was suggested by Batchelor and Christie *(2)* for the dehydration of freshly harvested in-shell walnuts. These workers observed that irreversible flavor and quality losses occurred above the critical drying temperature. In-shell kernels are generally considered more stable than shelled or broken kernels *(12)*. Since the critical temperature decreases with increasing moisture content *(2)*, it was anticipated that drying temperatures above 110° F might be especially disadvantageous for broken kernels obtained from the Romberg process. Therefore, preliminary drying experiments with the belt-trough dryer were conducted using air temperatures of 130° to 145° F in order to maintain the kernel temperatures below 110° F throughout the drying period. However, under these conditions, drying times were excessively long. A second series of preliminary experiments were conducted at more elevated temperatures. Although kernel temperatures exceeded the critical 110° F for short periods of time, no immediate off-flavors were detected in the dried kernels. It was concluded that walnut kernels may be exposed to temperatures considerably in excess of 110° F for short periods without affecting their initial quality. The present paper describes in more detail a third series of studies on drying high-moisture kernels with the belt-trough dryer at inlet air temperatures of from 160° to 255° F.

## EXPERIMENTAL

**Materials.** Moist kernels containing 15 to 20% moisture were obtained from freshly prepared batches of rehydrated shell and kernel mixtures. Variations in initial moisture content of different batches were due to variations in the amount of surface moisture removed by centrifugation and drainage following the rinse operation. The kernels represented mixed varieties, and ranged in size from small pinhead-sized pieces to half kernels. Commercially, this grade of kernels is called "syruper's pieces."

**Equipment.** The experimental laboratory model belt-trough dryer described by Lowe *et al (5)* was employed. The belt speed was operated at 7.5 fpm. Incoming air velocity averaged 3500 fpm, with a static pressure ranging from 1.25 to 1.5 in wg measured at the hot air inlet. Except where noted, the bed depth was 4 in representing 100 lb of moist kernels. The belt was operated in a horizontal position for the batch drying experiments. Markers incorporated in the bed indicated no significant lateral movement among the rotating kernels.

## METHODS

*Moisture.* Kernel moisture was estimated by the Moisture Balance procedure described by Rockland *(7)*.

*Oxygen absorption.* Oxygen was estimated as described by Swarthout *et al (11)* in the headspace gases of samples held at 95° F for 80 days after dehydration.

*Kernel evaluation.* Taste and quality evaluations were determined subjectively by a panel of about 10 persons including production plant workers and supervisors, as well as the laboratory staff, all of whom were experienced in estimating kernel quality.

## PROCEDURE

The air stream of the belt-trough dryer was adjusted to the desired temperature by means of an automatic temperature recorder-controller, and checked by a bimetallic dial thermometer located in the throat of the air inlet tube. A vibrator type dispenser and small moving-belt conveyor were employed to load the dryer rapidly, uniformly and with minimum abrasion to the kernels. A bed depth of 4 in was obtained with 100 lb of kernels spread over the 8 sq ft surface. In one experiment, a bed depth of about 7 in was obtained with 180 lb of moist kernels.

Wet and dry bulb temperature readings were recorded periodically at 6 positions over the rotating bed by means of a calibrated copper-constantan thermocouple connected to a temperature indicating galvanometer. The exhaust air temperatures and relative humidities were uniform over the horizontal bed. The relative humidities of the exhaust air streams varied between 4 to 14% and averaged about 8% at the end of the drying period. Kernel temperatures were obtained with the same equipment by inserting thermocouple leads into the center of large kernels picked from the center surface of the rotating bed. Kernel temperatures presented in the Tables represent the average of 10 individual determinations taken within a 2 min period. Approximately 200 g samples were taken from the top 2 in of the center of the moving bed for moisture analyses. Kernels were placed immediately in glass jars, sealed with rubber-gasketed lids, and allowed to cool and equilibrate before moisture estimations were performed. Kernel quality was assessed during dehydration to determine if toasted or other off-flavors and -odors developed in the heated kernels. No marked off-flavors or -odors were detected during dehydration except at 255° F when a definite toasted flavor developed within 15 min or after prolonged drying at 200° to 210° F. Quality evaluations were conducted on the warm kernels after the completion of each experiment as well as 24 hr later. Final quality evaluations were conducted on samples from each experiment after storage in the dark at 95°F for 80 days in sealed glass jars.

## RESULTS AND DISCUSSION

At a belt speed of 7.5 fpm kernels were rotated gently and with minimum apparent abrasion, although a slight polishing effect was observed. No significant loss of fines or clogging of the wire screen belt was encountered. An unanticipated benefit was obtained by the removal of loose, oil-soaked pellicle and chaff which was blown off the surface of the bed by the air stream, and which resulted in improved kernel appearance.

Single-stage dehydration experiments were performed at 180°, 190°, 200° and 255° F on a 4 in bed, and at 190° F on a 7 in bed. Two-stage drying was investigated using a preliminary drying temperature of 160° F and a final temperature of 200° F (160°/200° F), as well as using a preliminary drying temperature of 180° F and a final drying temperature of 210° F (180°/210° F). Variations in kernel moisture content and temperatures as a function of dehydration temperature and time are presented in Table 1. Quality changes observed during dehydration and after storage of kernels for 80 days at 95° F as well as

**TABLE 1**

**Dehydration of walnut kernels on a belt-trough dryer at 180° to 255° F [1]**

| Drying time | Drying temperature, °F | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 180° | | 190° (7" bed) | | 190° | | 200° | | 160°/200° | | 180°/210° | | 255° | |
| | Kernel temp | Moisture content | Kernel temp | Moisture content | Kernel temp | Moisture content | Kernel temp | Moisture content | Kernel temp | Moisture content | Kernel temp | Moisture content | Kernel temp | Moisture content |
| *min* | °F. | % | °F. | % | °F. | % | °F. | % | °F. | % | °F. | % | °F. | % |
| 0 | 80 | 17.3 | 80 | 17.7 | 80 | 16.6 | 80 | 15.5 | 80 | 15.5 | 80 | 16.6 | 80 | 19.9 |
| 5 | 115 | 12.6 | 109 | 13.1 | 118 | 11.2 | 121 | 9.9 | 115 | 12.4 | 114 | 12.1 | 160 est | 14.7 |
| 10 | 126 | 10.2 | 118 | 10.7 | 127 | 8.3 | 135 | 8.1 | 127 | 10.1 | 125 | 9.7 | 173 | 9.8 |
| 15 | 131 | 8.8 | 126 | 9.3 | 132 | 7.2 | 140 | 7.0 | 135 | 8.6 | 127 | 8.0 | 176 | 7.4 |
| 20 | 133 | 7.9 | 132 | 8.3 | 133 | 6.5 | 142 | 6.2 | 140 | 7.2 | 131 | 6.8 | 178 | 5.8 |
| 25 | 133 | 7.2 | 136 | 7.5 | 134 | 5.9 | 142 | 5.6 | 142 | 6.2 | 132 | 6.0 | 179 | 4.4 |
| 30 | 133 | 6.7 | 138 | 6.9 | 135 | 5.5 | 142 | 5.2 | 142 | 5.6 | 132 | 5.2 | 180 | 3.5 |
| 35 | 133 | 6.3 | 138 | 6.4 | 136 | 5.1 | 142 | 4.8 | 143 | 5.1 | 133 | 4.6 | ..... | ..... |
| 40 | 133 | 6.0 | 138 | 5.9 | 136 | 4.7 | 142 | 4.5 | 143 | 4.8 | 134 | 4.2 | ..... | ..... |
| 45 | 133 | 5.7 | 138 | 5.4 | 137 | 4.4 | 143 | 4.2 | 143 | 4.5 | 137 | 3.8 | ..... | ..... |
| 50 | 133 | 5.5 | 138 | 5.0 | 137 | 4.1 | 143 | 3.9 | 143 | 4.2 | 141 | 3.6 | ..... | ..... |
| 55 | 133 | 5.3 | 138 | 4.8 | 138 | 3.9 | 143 | 3.7 | 144 | 4.0 | 146 | 3.4 | ..... | ..... |
| 60 | 133 | 5.1 | 138 | 4.4 | 138 | 3.7 | 143 | 3.5 | 144 | 3.9 | 151 | 3.2 | ..... | ..... |
| 70 | 133 | 4.9 | 138 | 3.9 | 138 | 3.3 | 144 | 3.3 | 144 | 3.6 | ..... | ..... | ..... | ..... |
| 80 | ..... | ..... | 138 | 3.5 | 139 | 3.1 | | | 144 | 3.2 | ..... | ..... | ..... | ..... |
| 90 | ..... | ..... | 138 | 3.2 | ..... | ..... | 158 [2] | 2.8 | ..... | ..... | ..... | ..... | ..... | ..... |

[1] Dry-bulb temperature of inlet air stream at 3500 cfm through a 4 in bed containing 100 lb of moist kernels at a belt speed of 7.5 fpm. A bed depth of 7 in was obtained with 180 lb of moist kernels. Horizontal double line indicates time at which the inlet air temperature was raised.
[2] Air inlet temperature raised to 230° after 80 min at 200° F.

oxygen absorption measurements made after storage are shown in Table 2. Dehydration rates, expressed as the time required to reach optimum kernel moisture (3.5%) are presented in Table 3.

Severe kernel damage occurred during dehydration at 255° F. At the end of 30 min, kernels were literally "*roasted in their own oil*" giving the kernels an unobjectionable but distinctly toasted flavor. A temperature of 200° F was the highest that could be employed for single-stage dehydration without causing significant flavor changes while drying to the optimum moisture. However, excessive heating at 190° F on a 4 in bed also induced a slight flavor change and decreased kernel stability. No significant off-flavor or reduced stability was obeserved for kernels dried at 180° F on a 4 in bed or 190° F on a 7 in bed. Since heat damage to kernels increased with increasing moisture content, prolonged drying at 200° F was tolerated during a two-stage drying process in which first-stage drying to about 9% moisture was conducted at 160° F. Kernels were also subjected to a temperature of 210° F for 25 min after first-stage drying at 180° F for a like period without inducing any apparent initial flavor changes. However, prolonged heating and moisture reduction of kernels below 3.5% resulted in a slight quality loss which was amplified after holding for 80 days at an incubation temperature of 95° F in a sealed glass jar. During the same holding period, kernels dried at 190° F on a 7 in bed and at 160°/200° F on a 4 in bed remained acceptable.

In general, kernel temperatures remained 25° to 75° F below the air inlet temperatures. At moisture levels between 7 and 3.5%, kernels could be maintained at 140° to 145° F without affecting apparent initial quality or stability. However, even abbreviated periods at moisture levels below about 3.3% and kernel temperatures of 150° F produced toasted or other off-flavors and lowered stability. At kernel temperatures of 160° to 170° F serious off-flavors developed within 10 min in kernels at high moisture levels.

Batchelor and Christie *(2)* reported the development of off-flavors in in-shell walnuts dried at inlet air temperatures of 130° F and above when the initial

**TABLE 2**

**Kernel quality changes during and after dehydration on the belt-trough dryer [1]**

| Characteristic | | | Drying temperature, °F | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 180° | 190° (7" bed) | 190° | 200° | 160°/200° | 180°/210° | 255° |
| Kernel flavor evaluation | During dehydration to 3.5% moisture | | Good | Good | Good | Good-fair | Good | Good | Toasted, overheated |
| | At lowest moisture attained [3] | Initial | Good-fair (4.9%) | Good-fair (3.2%) | Fair-poor (3.1%) | Fair-poor (2.8%) | Fair (3.2%) | Fair-poor (3.2%) | ..... |
| | | After 80 days at 95° F | Fair | Fair | Slightly stale | Slightly stale | Fair | Slightly stale | ..... |
| Oxygen absorption [2] % | At lowest moisture attained | After 80 days at 95° F | 18 | 21 | 26 | 20 | 20 | 30 | ..... |

[1] See footnote [1], Table 1.
[2] Amount of oxygen absorbed from the headspace gas contained in a 12 oz sealed glass jar containing 100 g kernels (10); equal to [(20.93 — % $O_2$ in headspace gas) x 100/20.93].
[3] Lowest moisture attained is given in parentheses.

TABLE 3

**Dehydration rates at various temperatures on belt-trough dryer, expressed as time required to reach optimum (3.5%) moisture**

| Drying temperature | Kernel bed depth | Final kernel temperature | Total time to reach 3.5% moisture[2] |
|---|---|---|---|
| °F | in | °F | min |
| 180 | 4 | 135 est | 100 |
| 190 | 7 | 138 | 80 |
| 190 | 4 | 138 | 65 |
| 200 | 4 | 143 | 60 |
| 160 (15)/200[1] | 4 | 144 | 70 |
| 180 (25)/210[1] | 4 | 144 | 50 |
| 255 | 4 | 178 | 30 |

[1] Two-stage drying. The first number indicates the first stage drying temperature followed by time in minutes at this temperature given in parentheses.

[2] Interpolated from smoothed dehydration rate curves.

moisture content of the whole nut was 29%, equivalent to about 10% moisture in the kernel. However, the nuts were dried for periods of from 24 to 48 hr and the kernel temperatures presumably approached the air temperature toward the end of the drying period. It is apparent from the present studies that kernels may be exposed for short periods to drying temperatures 100° F higher than the critical temperature of 110° F reported by Batchelor and Christie if a highly efficient dehydration process is employed and kernel temperatures do not exceed 145° F. Initial dehydration rates were extremely rapid in the belt-trough dryer. Up to one half of the kernel moisture was removed within 20 min at temperatures of 160° F and above without loss in flavor or quality if the kernel temperatures did not exceed 135° to 140° F. Maximum drying efficiency and minimum quality loss were observed at 190° to 200° F for single-stage drying. The most efficient drying was obtained using a two-stage drying process in which kernels were dried on a 4 in bed for 25 min at 180° F followed by an equal time at 210° F. However, extended drying at 210° F reduced the moisture level to 3.2% and imparted a slightly toasted flavor and reduced stability.

## SUMMARY

Dehydration rates have been estimated for high-moisture walnut kernels in a laboratory model belt-trough dryer at temperatures ranging from 160° to 255° F. Kernel moistures were reduced to the optimum moisture range (3.5 ± 0.3%) from 15 to 20% within 65 minutes during single-stage drying at 190° F and within 50 minutes during two-stage drying for about equal periods at 180° and 210° F, respectively. Under these conditions no significant off-flavors developed during dehydration. However, excessive drying to 3.2% moisture or lower produced a slightly toasted flavor and more rapid deterioration, especially at the higher dehydration temperatures. At an air inlet temperature of 255° F kernels developed a distinct toasted flavor within 15 minutes although the kernel moisture level was above 5%.

These investigations corroborate Batchelor and Christie's *(2)* observations that the critical temperature for walnuts decreases with increasing moisture content. These investigators also observed significant flavor changes or accelerated rancidity development for in-shell walnuts after bin-drying for 24 to 48 hours at 120° F and higher. However, the present authors have observed no initial impairment of kernel quality at drying temperatures as high as 210° F and kernel temperatures up to 145° F after the short drying periods required by the belt-trough dryer.

Kernels dried on the belt-trough dryer developed a slight polish although kernel abrasion appeared to be minimized. Improved kernel appearance may have been due in part to removal of loose oil-soaked pellicle, chaff and fines which were removed by updraft in the warm air stream penetrating the kernel bed. No significant loss of fines or clogging of the screen belt were observed.

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# Size of Sample for Maturity Determination of Canning Corn*

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A VARIETY OF METHODS has been used for the determination of maturity and quality of sweet corn *(1, 4, 5, 6, 7, 8)*. The size of the sample used in the various instruments and techniques varied from 10 g for moisture measurement with the Steinlite *(6)*, to 6 lb for measurement of specific gravity *(4)*. Because the corn samples came from uniform fields, it could be assumed that the factor of field variability was small, so that the quality measurements obtained on a small sub-sample would be representative of the entire field plot. In those cases where the quality indices of raw and processed corn were compared, the difficulty of variability between the small sample for instrument measurement and the larger sample for processing was avoided by grading each ear of corn. In this way the experimental material could be arranged in definite maturity ranges *(7)*.

Under commercial conditions, the most serious problem related to sampling procedure is that of size of sample which will give a maturity estimate representative of the corn population sampled. Scott *et al (9)* determined the maturity index of a load of corn, as received at the cannery, by using a 40-lb sample of unhusked ears, from which a 2-lb sub-sample was drawn for measurement of the refractive index. Similarly, Cameron *et al (3)* used a 40-lb sample of unhusked corn per load when comparing the thumbnail, Brown-Duval, semi-automatic moisture test and specific gravity test. Berth and Weckel *(2)* suggested that 30 ears would be sufficient to indicate the average maturity of a load of corn.

Selecting optimum maturity levels in sweet corn for processing necessitates periodic sampling of the corn prior to harvest. Since most instrument techniques use very small samples, the attainment of a representative field sample is extremely important. Although considerable work has been done in the maturity testing of corn, it appears that information with respect to sampling methods under commercial conditions is still lacking. The present study was designed to fulfill the first requirement of estimating the optimum sample size in order to obtain quality measurements within definite limits of accuracy.

## METHODS

A commercial field, 540 ft x 615 ft in Eastern Ontario was planted with the variety Tendermost. The field was reasonably uniform in appearance and typical of most of those in the area. The field was planted on April 3, and normal farm practices were followed.

The maximum number of Steinlite determinations which could be made in a single day was 60, and since it was undesirable to hold over any ears, the following sampling scheme was adopted. During the week when optimum maturity of the field was expected, 60 ears of corn were collected 3 times at 2-day intervals. Positions were selected each day at random and 6 ears picked at each position. The randomization of the positions was done beforehand by determining position coordinates from a set of random numbers and marked in the field by pacing off along the length of the rows and counting rows in the other direction. A different randomization was used for each date of harvest. No randomization process was used in selecting the 6 ears at each location but no particular selection of ears was made except to avoid obviously off-type plants. A single ear was harvested from each plant. The ears were numbered according to position and order of picking and stored at 32° F until the Steinlite determinations were made on each single ear, and the sixty completed on the same day as harvested.

The analysis of variance of the data for each harvest date was set up as shown in Table 1.

TABLE 1

**Analysis of variance of moisture determination data for each harvest date**

| Source | Degrees of freedom | Composition of mean squares |
|---|---|---|
| Positions | $(p-1)$ | $\sigma_e^2 + S\,\sigma_p^2$ |
| Sub-samples | $p(s-1)$ | $\sigma_e^2$ |

where

p = number of positions.

s = number of determinations (ears) at each position.

$\sigma_e^2$ = variance component due to determinations within positions.

$\sigma_p^2$ = variance component due to positions.

From the algebraic composition of the two mean squares, the estimated values of the components were calculated. This was done for the data from each harvest date. It should be pointed out here that $\sigma_e^2$ includes variance due to several factors, i.e. ear to ear and plant to plant, Steinlite method itself, blenders and operators. In this plan, there is no way of separating out variance due to these factors.

Having estimated values for the two components, the estimated variance of a mean can then be determined for any sampling scheme (any combination of positions and number of determinations at each position). The estimated variance of a mean can be expressed as

$$\text{var}\ \bar{x} = \frac{\sigma_p^2}{p} + \frac{\sigma_e^2}{ps}$$

where p and s are the number of positions and the number of determinations at each position, respectively. This method of analysis has been found useful where single determinations on a sample made up of the kernels from a single ear are made.

In the case where single Steinlite determinations are made on a composite sample made up of the kernels from a number of ears, the estimated variance of a mean would be:

$$\text{var}\ \bar{x} = \frac{\sigma_p^2 + \sigma_e^2}{p}$$

* Joint contribution from the Statistical Research Service and the Plant Research Institute (Contribution No. 86), Research Branch, Canada Department of Agriculture, Ottawa.

This formula overestimates var $\bar{x}$, the actual value being somewhere between this value and that obtained from the previous formula for the same number of ears as form the composite. The exact value of var $\bar{x}$ cannot be determined since it depends upon the relative contributions of the various factors making up $\sigma_s^2$. However, it may be used for practical purposes as it would give results which are always on the safe side.

The reliability of a mean for any sampling scheme can best be expressed in terms of confidence limits which are computed as $t_{.05} \sqrt{\text{var } \bar{x}}$ where $t_{.05}$ is the tabulated value of Students' t at the 5% level for $(p-1)$ degrees of freedom and $\sqrt{\text{var } \bar{x}}$ is the standard error of a mean. At the 5% level, the probability is 95% that the confidence limits will have values which include between them the true mean. In calculating the confidence limits, average values of $\sigma_p^2$ and $\sigma_s^2$ over the 3 harvest dates were used. The values shown for composite samples assume a single Steinlite determination made on a composite sample from six ears at each position.

Following the previously outlined procedure an analysis of variance was carried out on the data from 10 positions with 6 Steinlite determinations at each position for each harvest date. Table 2 shows the analysis for the data from the first harvest.

**TABLE 2**

**Analysis of variance, ears harvested Sept. 8**

| Source | Degrees of freedom | Mean square | Composition of mean squares |
|---|---|---|---|
| Position | 9 | 33.2637 | $\sigma_s^2 + \sigma_p^2$ |
| Ears in Positions | 50 | 7.8785 | $\sigma_s^2$ |
| Total | 59 | | |

Equating the composition of mean squares to the values found in the analysis the values of $\sigma_s^2$ and $\sigma_p^2$ were found as:

$$\sigma_s^2 = 7.8785$$
$$\sigma_p^2 = 4.2309$$

These components were determined for data from three harvest dates and the average values calculated as:

$$\sigma_s^2 = 7.7196$$
$$\sigma_p^2 = 3.5936$$

Using the average values for $\sigma_s^2$ and $\sigma_p^2$, the variance of a mean based upon Steinlite readings on six individual ears of corn at each of ten positions is

$$\text{var } \bar{x} = \frac{3.5936}{10} + \frac{7.7196}{60} = .4880$$

Confidence Limits are then $\pm \sqrt{.4880} \times 2.262 = \pm 1.58$ where 2.262 is the tabulated value of "t" at the .05 probability level for $(p-1) = 9$ degrees of freedom.

The variance of a mean based upon one Steinlite determination on a composite sample of ears at each of ten positions would be estimated as

$$\text{var } \bar{x} = \frac{3.5936 + 7.7196}{10} = 1.1313$$

The Confidence Limits then become $\pm \sqrt{1.1313} \times 2.262 = \pm 2.41$.

## DISCUSSION OF RESULTS

Table 3 presents the confidence limits for various sampling schemes calculated from the field data as previously described. Confidence limits are shown for positions varying from 2 to 10 with 1 to 6 determinations at each position. The right hand column shows these limits for 2 to 10 positions with a single determination made on a single ear or on a composite sample from a number of ears of corn at each position.

This table illustrates the effects of decreasing the size of sample on the accuracy of a mean. This is shown by the rapid increase in the size of the confidence limits with smaller samples, particularly when the total number of ears is collected at less than 5 positions in the field. While the confidence limits have increased by only .74 by decreasing the number of positions from 10 to 6 (6 determinations at each position), there is an increase of 17.11 when the number of positions is decreased from 5 to 2. A similar pattern if followed in the case of single determinations on either single ears or composite samples of a number of ears.

**TABLE 3**

**Confidence limits for various sampling schemes**

| No of positions | No of ears or determinations at each position | | | | | |
|---|---|---|---|---|---|---|
| | 6 | 5 | 4 | 3 | 2 | 1 |
| 10 | 1.58 | 1.62 | 1.68 | 1.78 | 1.95 | 2.41 |
| 9 | 1.70 | 1.74 | 1.79 | 1.91 | 2.10 | 2.59 |
| 8 | 1.85 | 1.90 | 1.97 | 2.08 | 2.28 | 2.81 |
| 7 | 2.04 | 2.10 | 2.17 | 2.30 | 2.53 | 3.11 |
| 6 | 2.32 | 2.38 | 2.47 | 2.61 | 2.87 | 3.53 |
| 5 | 2.74 | 2.81 | 2.92 | 3.08 | 3.39 | 4.18 |
| 4 | 3.51 | 3.61 | 3.74 | 3.95 | 4.34 | 5.35 |
| 3 | 5.49 | 5.63 | 5.84 | 6.17 | 6.78 | 8.36 |
| 2 | 19.85 | 20.40 | 21.11 | 22.31 | 24.53 | 30.22 |

Changes in the number of positions selected had a greater influence upon the confidence limits, hence on the accuracy of a mean, than changes in the number of ears harvested at each position. This is apparent from the relatively rapid expansion in the confidence limits as the number of positions is decreased, compared to a much smaller increase as the number of ears at each position is reduced. From this it becomes obvious that the most efficient way of increasing the accuracy of sampling is by increasing the number of positions in the field at which ears are selected for testing.

In practice a sampling scheme whereby a single Steinlite determination is made on a composite sample at each position in the field would undoubtedly be the most satisfactory because of the time required in the Steinlite procedure. The selection of a particular scheme would depend upon two main considerations, the desired degree of accuracy and the amount of time, and therefore cost, which could be allowed per estimate of mean moisture content of a field.

For example, suppose that a maximum of 10 Steinlite determinations at a particular field was the limit of sampling possible. Two possible sampling schemes might be considered. The first scheme involves Steinlite determinations on each of 2 ears collected from 5 random positions in the field. Confidence limits, from Table 3 are found to be $\pm 3.39$ so that the true mean moisture content may be expected to lie in the range "observed mean $\pm 3.39$" unless a 1 in 20 chance has occurred. The alternative scheme involves one Steinlite determination made on a composite sample at each of 10 random positions. Confidence limits are then found $\pm 2.41$, so that the mean moisture content is determined somewhat more accurately by this scheme. Approximately the same accuracy as in the first scheme using single determinations on composite samples could be obtained with only 7 actual Steinlite

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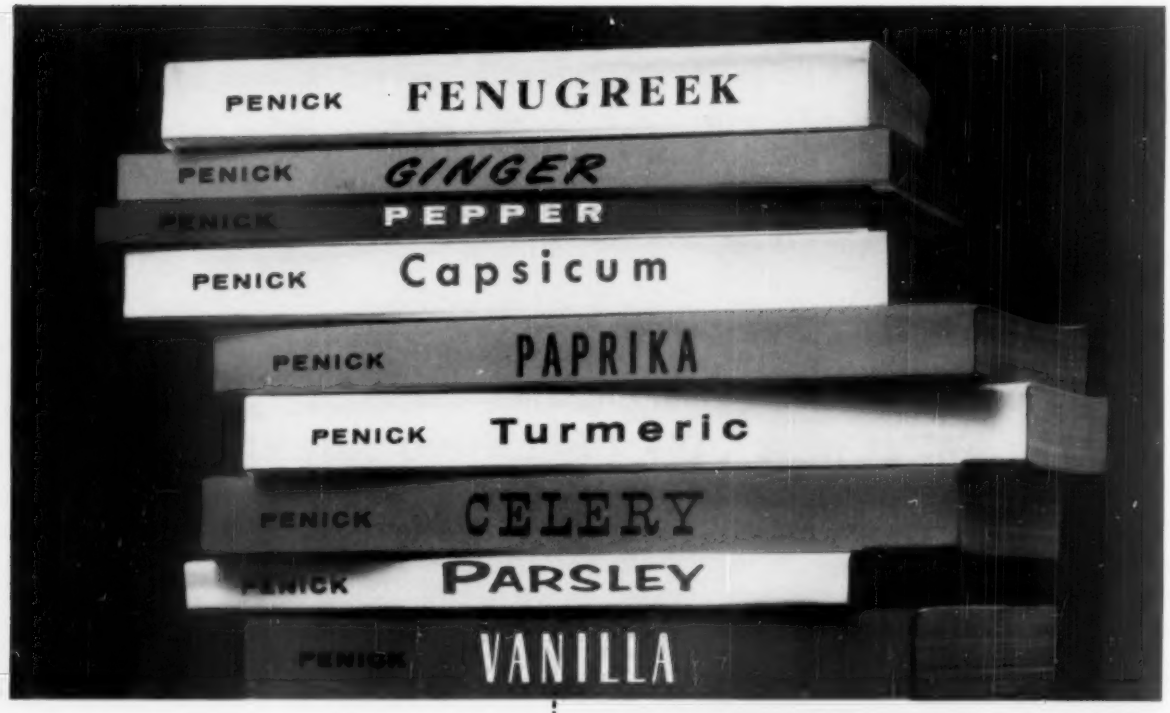


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determinations since the confidence limits for this number of positions are ±3.11.

The actual limits within which the mean moisture content must lie are determined by the desired quality of the processed product. Suppose that the limits of moisture content acceptable to the processor were 70.0% and 80.0%. This means that the true mean moisture content of the field must lie somewhere between these limits. The confidence limits of the observed mean will depend upon the sampling scheme adopted. If it were decided that 10 Steinlite determinations could be made on composite samples, Table 3 shows confidence limits of ±2.41 for this scheme. The lower limit of the observed mean would then be 70.0 + 2.41 = 72.41% while the upper limit would be 80.0 − 2.41 = 77.59%. The observed mean would have to lie in the range 72.41–77.59% to be reasonably sure that the true mean moisture content is within the limits prescribed. It is apparent that if the size of sample is reduced, the confidence limits would be increased, so that the range within which the observed mean must lie is narrowed. If sampling were reduced to 6 determinations on composite samples, the confidence limits would be ±3.53 and the observed mean would then have to lie in the range 73.53–76.47%. If estimates of mean moisture content are determined from smaller samples, a field would therefore have to be sampled on more days to be reasonably sure that it was harvested at the desired stage of maturity.

## SUMMARY

This study represents an attempt to devise suitable sampling procedures for determining the mean moisture content of a field of corn using the Steinlite method of determination. Two factors influencing variability within the field have been estimated and have shown that position in the field was considerably more important than ear to ear variation (including determination to determination variability, etc.). Using these estimates the accuracy, expressed as confidence limits, for a number of different schemes has been presented. The investigation has shown that very inaccurate estimates of the mean moisture content of a field of corn may result when an estimate is based upon too few samples. Increasing the number of positions in the field at which ears are harvested appears to be much more efficient in improving accuracy than increasing the number of ears at any one position. While it is recognized that these results apply to these specific data, the main considerations may be useful in deciding upon a sampling scheme under all ordinary conditions.

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# Radiation Resistance of the Natural Bacterial Flora of Cured Ham[a]

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(Manuscript received May 31, 1960)

USE OF IONIZING RADIATION from spent fuel rods and linear accelerators has been proposed as a method of destroying the microbial population of foodstuffs to render the packaged or canned product commercially sterile. In order for a food process to be economically feasible in industry the level of spoilage must be extremely low. To preserve the nutritive properties of canned foods it would be desirable to use the minimum process schedule and still retain the necessary low level of spoilage, provided, of course, the process is adequate to destroy all potential food poisoning organisms.

The purpose of this study was to determine the level of irradiation necessary to destroy and/or inactivate spores and vegetative bacterial forms naturally occurring in meat. It has been previously determined by Anderson *et al (1)* that a micrococcus was able to survive high levels of radiation. This vegetative culture was originally isolated from meat. Previous work on irradiation resistance of spores by Morgan and Reed *(7)* has suggested that *Clostridium botulinum* spores are more resistant than Putrefactive Anaerobe 3679 spores. This is a reverse relationship compared with the relative resistance to heat, i.e., PA 3679 spores are much more heat resistant than *Cl. botulinum* spores. Drake *et al (4)* studied the effect of heat and irradiation on the microflora of canned ham. They concluded that combination methods of heat processing and irradiation did not appear to have any particular advantage over present methods of heat processing followed by refrigerated storage. Results on irradiation of chicken carcasses by Coleby, Ingram and Shepherd *(3)* indicated severe flavor changes in this product when stored for extended periods of time.

## EXPERIMENTAL PROCEDURES

Three separate experiments were conducted to study bacterial resistance to irradiation. The ham for the first experiment was a portion of the composite cured ham sample reported by Brown, Vinton and Gross *(2)*. Composite ham for the second experiment was obtained by taking samples from the Ottumwa plant canning lines twice a day for 10 days during the month of September 1959. The meat used in experiment 3 was raw pork collected over a 10-day period and used as a composite sample. All samples were collected fresh, sampled for initial counts, immediately frozen and held at 0° F until ready for mixing and canning. A laboratory mixer was used to mix the samples and the meat was immediately filled into 208 x 109 cans and sealed. Each can contained approximately 100 g of meat. All cans were frozen at 0° F and held at this temperature before irradiation, irradiated at ambient temperature and immediately refrozen.

The cans of meat were irradiated at W. F. and John Barnes Company, Rockford, Illinois. This electron beam source was checked for dosimetry by representatives of the Quartermaster Food and Container Institute for the Armed Forces, Chicago. The reported doses represent levels of irradiation at the center of the can of meat. In order to determine whether or not differences exist in bacterial destruction between a linear accelerator and gamma source one half of the cans from experiment 2 were irradiated at the Argonne National Laboratory, Lemont, Illinois. The gamma source (spent fuel rods) dosimetry was also carefully determined by the Quartermaster Food and Container Institute for the Armed Forces.

Cans were defrosted with tap water at a temperature of 76° F until a meat temperature of 40° F was reached. The cans to be incubated were placed immediately in the incubator at 30° C or 26° C and observed 3 times a week for evidence of spoilage. Microscopic examination was made of the spoilage types.

Total counts were made by aseptically transferring 10 g of meat from each can to 90 ml of sterile distilled water in a blender. The sample was emulsified and dilutions made to duplicate plates using Trytone Glucose Yeast Extract Agar for total counts. The recovery media for anaerobic counts was Eugon agar suggested by Wheaton *et al (9)* as the best media for recovery of irradiated PA spores. Heat resistance determinations were made using the end point technique described by Stumbo *et al (8)*.

## RESULTS AND DISCUSSION

Our initial experiment was designed to study the effect of radiation on the natural flora of a composite ham sample. The 208 x 109 cans were opened immediately after irradiation and counts made. Duplicate plate counts were made in TGYE to determine destruction of aerobic cells. Anaerobic counts with Eugon agar were made using Halvorson and Ziegler's *(6)* most probable number technique to determine survivors. Individual colonies were examined microscopically to determine surviving types. Thermal resistance determinations were made on a 1-g sample. These results are presented in Table 1.

Plate counts from Table 1 indicate the destruction between 0.16 and 0.32 megarad of all organisms able to grow aerobically. Anaerobic spores remained viable in extremely small numbers after the maximum radiation level of 5.12 megarad. A coccus form was also able to survive high levels of irradiation when survivors were checked microscopically.

Thermal resistance values shown in Table 1 indicate the presence of PA 3679 since small numbers of spores of this organism are able to survive extremely

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[a] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned number 1067 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

[b] Presented at the Twentieth Annual Meeting of the Institute of Food Technologists, San Francisco, California, May 17, 1960.

TABLE 1

**The effect of different levels of radiation on the destruction of the natural flora of a composite ham sample**

| Radiation level (megarad) | Plate count bacteria/g | Anaerobic count bacteria/g | Thermal resistance ($F_0$ units) |
|---|---|---|---|
| 0 | 26,000 | 540 | 3.0 |
| .02 | 8,000 | 275 | 3.0 |
| .04 | 5,000 | 275 | 0.6 |
| .08 | 1,250 | 275 | 0.6 |
| .16 | 105 | 290 | 0.2 |
| .32 | .....* | 80 | 0.2 |
| .64 | .....* | 110 | 0.2 |
| 1.28 | .....* | 20 | 0.6 |
| 2.56 | .....* | 20 | .... |
| 5.12 | .....* | 30 | 0.6 |

* Less than 10 bacteria/g.

high levels of heat. Microscopic examination and later identification of this organism confirmed its identity as the S-2 strain of PA 3679.

The composite ham sample was inoculated with 1000 S-2 PA 3679 spores per gram then a duplicate set of cans filled for radiation. The same handling procedure was followed and results of survivor counts can be observed in Table 2. The counts are higher for

TABLE 2

**The effect of different radiation levels on the destruction of the natural flora of a composite ham sample with 1000 S-2 PA 3679 spores added per gram**

| Radiation level (megarad) | Plate count bacteria/g | Anaerobic count bacteria/g | Thermal resistance ($F_0$ units) |
|---|---|---|---|
| 0 | 26,000 | 1540 | 3.0 |
| .02 | 5,000 | 2300 | 1.0 |
| .04 | 1,000 | 1000 | 1.0 |
| .08 | 300 | 160 | 1.0 |
| .16 | .....* | 1000 | 1.0 |
| .32 | .....* | 1000 | 1.0 |
| .64 | .....* | 377 | 1.0 |
| 1.28 | .....* | 275 | 1.0 |
| 2.56 | .....* | 125 | 1.0 |
| 5.12 | .....* | 40 | 0.6 |

* Less than 10 bacteria/g.

surviving anaerobic cells as would be expected. The thermal resistance values were slightly higher and more uniform with the added spores present in the meat.

The same meat was used to repeat the first experiment shown in Tables 1 and 2 using both the electron beam source and a gamma source. Irradiation dosage was changed to levels of 0, 1.0, 3.0, 5.0 and 7.0 megarad. Essentially the same results were found in this repeat experiment using the composite ham sample. No differences in destruction of PA spores could be detected between the electron beam and gamma source.

A rate of destruction with heat of the natural spores in this composite ham sample is shown in Table 3. These data show that spores of this strain of PA have a marked resistance to heat as well as irradiation when present in low numbers. The coccus able to withstand high levels of irradiation in experiment 1 was grown in pure culture and its resistance to heat found to be very low (was not able to survive 1 minute at 250° F). A gamma radiation resistant micrococcus isolated from food by Duggan *et al (5)* was also resistant to ultra violet light irradiation. These results suggest that differences in the ability of vegetative microorganisms to survive high levels of heat does not mean

TABLE 3

**Rate of destruction of the natural spores present in the composite ham sample**

| Heating level $F_0$ units (Equiv min at 250°) | Anaerobic count after heating |
|---|---|
| 0 | 540 |
| .01 | 428 |
| .05 | 428 |
| .20 | 79 |
| .60 | 62 |
| 1.00 | 2 |
| 1.50 | 0 |
| 2.00 | 0 |

that these same organisms can survive high levels of irradiation.

The second experiment was conducted to determine the ability of surviving spores and/or vegetative cells to grow and produce visible spoilage in the incubated cans after irradiation at different levels. The ham was ground, filled into 208 x 109 cans and frozen before shipment to the irradiation sites. A duplicate set of cans was prepared with 200 PA spores/gram added to the normal flora of the cured ham.

One set of cans was counted immediately after receiving the irradiated meat. Results are shown in Table 4 for the uninoculated meat and Table 5 for the inoculated samples. These data again indicate low numbers of organisms able to survive the irradiation treatment. As an additional method to detect anaerobic spores a sample was aseptically removed from the cans and transferred to glucose brain broth bottles for 48 hr incubation at 35° C. All samples shown in Table 4 were positive. The types of organisms surviving high levels of irradiation were the S-2 strain of PA 3679 and a coccus form that resists irradiation.

TABLE 4

**Survivors after irradiation of cured ham at different levels using an electron beam source and gamma source**

| Radiation level (megarad) | Electron beam source | | Gamma source | |
|---|---|---|---|---|
| | Plate count bacteria/g | Anaerobic count bacteria/g | Plate count bacteria/g | Anaerobic count bacteria/g |
| 0 | 17,000 | 240 | 17,000 | 240 |
| 0.5 | 60 | 240 | 135 | 130 |
| 1.0 | .... | 80 | 20 | 25 |
| 2.0 | 40 | 50 | 90 | 80 |
| 3.0 | 30 | 50 | 5 | 15 |
| 4.0 | 15 | 35 | 25 | 15 |
| 5.0 | 25 | 15 | 80 | 25 |
| 6.0 | 5 | 10 | 20 | 5 |
| 7.0 | 60 | 130 | 10 | 25 |
| 8.0 | 5 | 15 | 10 | 130 |

TABLE 5

**Survivors after irradiation of inoculated cured ham at different levels using an electron beam source and gamma source**

| Radiation level (megarad) | Electron beam source | | Gamma source | |
|---|---|---|---|---|
| | Plate count bacteria/g | Anaerobic count bacteria/g | Plate count bacteria/g | Anaerobic count bacteria/g |
| 0 | 17,000 | 1,000 | 17,000 | 1,000 |
| .5 | 130 | 100 | 20 | 110 |
| 1.0 | 140 | 330 | ....* | 6 |
| 2.0 | 370 | 1,000 | 40 | 16 |
| 3.0 | 40 | 210 | 10 | 16 |
| 4.0 | 120 | 23 | 20 | 20 |
| 5.0 | 20 | 5 | ....* | 11 |
| 6.0 | 10 | 2 | 70 | 22 |
| 7.0 | 30 | 5 | ....* | 27 |
| 8.0 | 30 | 7 | ....* | 17 |

* Less than 10 bacteria/g.

The surviving cocci in this experiment were able to grow aerobically. No significant differences were noted between irradiation sites; however, the inoculated set of cans showed a definite increase in PA count over the corresponding irradiation level of uninoculated meat.

A duplicate set of cans was incubated at 30° C for a period of 2 months to observe for signs of spoilage. The cans were examined 3 times a week and removed from the incubator when spoilage occurred.

Results presented in Table 6 for the uninoculated cans show that spores and in some cases vegetative

**TABLE 6**

**Bacterial counts of meat from Experiment 2 after incubation at 30° C for two months**

| Radiation level (megarad) | Electron beam source | | Gamma source | |
|---|---|---|---|---|
| | Plate count bacteria/g | Anaerobic count bacteria/g | Plate count bacteria/g | Anaerobic count bacteria/g |
| 0 | ....[1] | | ....[1] | ....[1] |
| 0.5 | 470 | 5,420 | ....* | 2 |
| 1.0 | 140 | 1,750 | ....* | 2 |
| 2.0 | 100 | 10,000 | ....* | 46 |
| 3.0 | 90 | 240 | ....* | 2 |
| 4.0 | 10 | 350 | ....* | 27 |
| 5.0 | ....* | 10 | ....* | 13 |
| 6.0 | 270 | 35 | ....* | 175 |
| 7.0 | ....[1] | ....[1] | ....* | 278 |
| 8.0 | ....* | 10 | ....* | 17 |

* Less than 10 bacteria/g.
[1] Spoiled after 6 days.

cells remain viable for 2 months in the meat. The cans, except controls, did not swell or show signs of spoilage after the incubation period of two months. The numbers of anaerobic bacterial cells increased with incubation. Normal levels of curing salts (salt, sugar, nitrate and nitrite) exert inhibitory effects on anaerobic spores in meat *(10)*. This inhibition is very pronounced in thermally processed meats of the luncheon meat type. The data in Table 6 indicate the possibility of a similar type inhibition with irradiation processing. Subcultures and microscopic examination of surviving cells showed the presence of *Clostridia* in all cans. Similar results were found in the cans containing meat inoculated with low numbers of S-2 PA 3679 spores.

A third experiment was designed to study the effect of leaving out the curing ingredients on germination and growth of spores after various levels of irradiation. Fresh raw pork was the meat for this experiment instead of cured ham. The cans were prepared in the same manner as described in preceding experiments. A single irradiation source was used since no significant differences were found between sources in the first two experiments. Five cans of pork were irradiated at each level to give a set for initial counts and cans for storage at different incubation temperatures. Again a duplicate set of cans was prepared with 300 PA spores per gram. Immediately after receipt of the cans from the electron beam source two sets were aseptically opened for initial counts. This information is summarized in Table 7.

Table 7 shows small numbers of organisms surviving irradiation. Microscopic examination of colonies again indicated the survival of *Clostridia*

**TABLE 7**

**Bacterial counts of raw pork after irradiation**

| Radiation level (megarad) | Raw pork | | Raw pork + 300 PA spores/g | |
|---|---|---|---|---|
| | Plate count bacteria/g | Anaerobic count bacteria/g | Plate count bacteria/g | Anaerobic count bacteria/g |
| 0 | 220,000 | 240 | 220,000 | 540 |
| 0.5 | 35 | 8 | 95 | 22 |
| 1.0 | ....* | 3 | 10 | 2 |
| 2.0 | 25 | 25 | 10 | 33 |
| 3.0 | ....* | < 1 | ....* | 13 |
| 4.0 | ....* | < 1 | ....* | 4 |
| 6.0 | ....* | 2 | ....* | 17 |
| 8.0 | 15 | 10 | ....* | 27 |
| 10.0 | ....* | 10 | 30 | 240 |
| 12.0 | ....* | 10 | ....* | 50 |

* Less than 10 bacteria/g.

(PA) and cocci. To determine the ability of surviving spores to grow and cause spoilage cans of raw pork and inoculated raw pork were incubated at 26° C and 30° C. Table 8 summarizes the data on raw pork.

**TABLE 8**

**Spoilage data on irradiated raw pork incubated at two temperatures**

| Radiation level (megarad) | Counts after 53 days incubation (bacteria/g) | | | |
|---|---|---|---|---|
| | 26° C | | 30° C | |
| | Plate count | Anaerobic count | Plate count | Anaerobic count |
| 0 | ....[1] | ....[1] | ....[3] | ....[3] |
| 0.5 | ....[2] | ....[2] | ....[4] | ....[4] |
| 1.0 | ....[2] | ....[2] | ....[4] | ....[4] |
| 2.0 | 500 | 70 | ....* | 9 |
| 3.0 | 40 | 70 | ....* | 349 |
| 4.0 | 30 | 220 | ....* | 4 |
| 6.0 | ....* | 1620 | ....* | 240 |
| 8.0 | ....* | 80 | ....* | 7 |
| 10.0 | ....* | 240 | ....* | 16 |
| 12.0 | 5000 | 220 | ....* | 4 |

* Less than 10 bacteria/g.
[1] Spoiled after 8 days.
[2] Spoiled after 10 days.
[3] Spoiled after 1 day.
[4] Spoiled after 2 days.

Spoilage was of a putrefactive nature and all cans through the level of 1.0 megarad spoiled. The cocci appear to diminish in numbers with incubation but the anaerobic count was higher indicating spore germination and growth. The inoculated raw pork results in Table 9 show even higher anaerobic counts especially at the lower incubation temperature (26° C). Perhaps more of the cans incubated at 26° C

**TABLE 9**

**Spoilage data on irradiated inoculated raw pork incubated at two temperatures**

| Radiation level (megarad) | Counts after 24 days incubation (bacteria/g) | | | |
|---|---|---|---|---|
| | 26° C | | 30° C | |
| | Plate count | Anaerobic count | Plate count | Anaerobic count |
| 0 | ....[1] | ....[1] | ....[2] | ....[2] |
| 0.5 | ....[1] | ....[1] | ....[2] | ....[2] |
| 1.0 | ....[1] | ....[1] | ....[2] | ....[2] |
| 2.0 | 410 | 540 | 590 | 20 |
| 3.0 | 500 | 1,620 | ....* | 5 |
| 4.0 | 10 | 540 | ....* | 40 |
| 6.0 | 2000 | 10,000 | 18 | 350 |
| 8.0 | 20 | 10,000 | 10 | 350 |
| 10.0 | 50 | 1,620 | 3100 | 540 |
| 12.0 | ....* | 220 | 260 | 30 |

* Less than 10 bacteria/g.
[1] Spoiled after 8 days.
[2] Spoiled after 1 day.
[3] Spoiled after 2 days.

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would have spoiled if the incubation period had been extended. Studies are underway to determine the spoilage level with extended storage periods.

The S-2 strain of PA 3679 was inoculated into sterile baby food and allowed to grow for 3 days at 37° C. This baby food was transferred to sterile 208 x 109 cans. The cans were sealed, frozen and irradiated at levels of 0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0 and 12 megarad. Immediately after radiation one half of the cans (0, 1, 3, 6 and 10 megarad) were checked for viability by aseptically transferring a 1-g portion of the baby food into brain broth bottles. All cans contained viable spores. The remaining cans were placed in the 30° C incubator for a period of 70 days. The contents were liquefied as determined by shaking the cans but no evidence of gas formation was noted. The meat from each can was counted in Eugon agar and the results are presented in Table 10. The spores were able to germinate and grow at 30° C and large numbers were found after incubation.

TABLE 10

Irradiated baby food counts after 70-days incubation at 30° C

| Radiation level (megarad) | Anaerobic count (bacteria/g) |
|---|---|
| 0.5 | $10^9$ |
| 2.0 | $10^9$ |
| 4.0 | $10^9$ |
| 8.0 | $10^8$ |
| 12.0 | $10^8$ |

Extensive damage to the enamel was encountered after incubation and can be seen in the photograph of Figure 1.

The anaerobic spores from each experiment that were able to survive high levels of irradiation were isolated, grown in pure culture and identified. In all cases they were the S-2 strain of PA 3679. Identification was made by fermentation of sugars, growth temperature, toxicity tests with guinea pigs and the extreme resistance to heat ($F_s9$) when grown in meat. A typical putrefactive odor was produced by these isolates.

Bacterial counts are extremely difficult to make with accuracy when small numbers of organisms are present therefore little significance can be placed on exact numbers in the tables. In all cases organisms were present when other measures were used to detect their presence such as heat resistance determinations and incubation at growth temperatures.

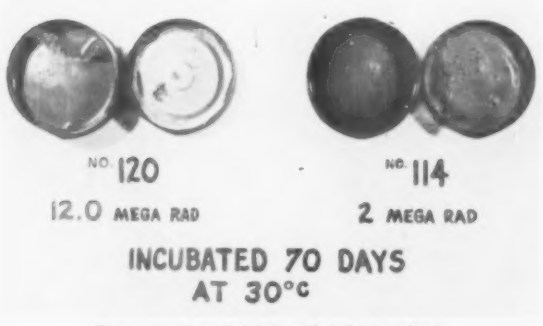


Figure 1. Inoculated irradiated baby food.

## SUMMARY

Three separate experiments were conducted to study the radiation resistance of the natural bacterial flora of canned ham. Most strains were extremely sensitive to irradiation and were destroyed at low levels. Two strains of cocci were able to survive high levels of radiation but were not studied extensively since in commercial processing they would be destroyed with low levels of heat. Small numbers of spores were able to survive high levels of radiation in cans of cured ham.

These spores were identified as the S-2 strain of PA 3679. If curing salts (salt, sugar, nitrate and nitrite) were present in normal amounts the ground ham did not spoil in the cans given at least 0.5 megarad irradiation when incubated at 26° C and 30° C for a period of two months.

The spores were able to grow and produce spoilage through a radiation level of one megarad, provided no curing salts were present in the meat. Counts after incubation indicated that the cells were increasing in numbers in cans of meat containing no curing salts.

No marked differences were found in destruction of bacteria between a gamma source and electron beam source of irradiation.

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# Anthocyanins. II.

## Action of Anthocyanin Pigments and Related Compounds on the Growth of Certain Microorganisms [a,b,c]

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(Manuscript received May 18, 1960)

THE CHEMICAL REACTIVITY of anthocyanin pigments in food has been recognized for 35 years or more *(6)*. Only within the past few years have anthocyanin pigments been demonstrated to possess activity toward bacteria *(8, 11, 14, 23, 24, 27, 38)*. Because of the relative ubiquity of anthocyanins in nature and the numerous forms in which they occur, this study was undertaken (a) to compare anthocyanin compounds for microbial activity, (b) to begin to correlate structure with activity, and (c) to learn whether heating affects activity.

Masquelier *(17, 23, 24)* and two of his doctoral students (Jensen and Lancepleine) at the University of Bordeaux were apparently the first to demonstrate that anthocyanin pigments may possess bactericidal activity. Prior investigators *(4, 20)* had not found activity. Jensen *(14)* credits Lancepleine with the idea of adding anthocyanin pigment to white wine to learn whether *Eberthella typhosa* would be more readily destroyed thereby. Jensen *(14)* herself established that a purified, partially-demethoxylated malvidin was bactericidal. Pratt, Powers, and Somaatmadja *(27)* observed that the monoglucosides of cyanidin, pelargonidin, and delphinidin decreased the lag phase of growth of certain bacteria but that these compounds also decreased the maximum growth attained. Recently, Masquelier *(22)* has stated that cyanidin and pelargonidin are not bactericidal.

### EXPERIMENTAL

**Anthocyanin compounds.** Five synthetic compounds were used. They were: apigeninidin-chloride; apigeninidin-chloride-4′-methyl-ether; 3′-methoxy-apigeninidin-chloride-4′-methyl-ether; 5-desoxy-apigeninidin-chloride-4′-methyl-ether; and 5-desoxy-3′-methoxy-apigeninidin-chloride-4′-methylether.

Natural anthocyanins contained in frozen strawberries, bottled Concord grape juice, and Pinot Noir wine were extracted with acidified butanol *(27)*. The pigments were separated and purified by paper chromatographic means and crystallized *in vacuo (27)*.

**Bacterial procedures.** Cultures of *Escherichia coli*, *Lactobacillus casei*, and *Staphylococcus aureus* were used. The cultures were maintained in tryptone-glucose-yeast (TGY) broth. The effect of the anthocyanin compounds or their breakdown products on the growth of the test organisms was determined by means of sensitivity discs. Tryptone-glucose-yeast agar was seeded with an 18-hr or 24-hr culture of the test organism (1% inoculum) and the inoculated medium was poured into Petri dishes. An aqueous, neutral solution (0.05 ml) of the compound being tested was added to sterile discs and allowed to dry. The discs were then placed upon the surface of the hardened agar. The plates were incubated at 37° C and examined at 24 and 48 hr for evidence of inhibition or stimulation of the test organism.

**Thermal degradation.** The synthetic compounds and the natural anthocyanins were heated for 30, 60, 90, and 120 min at 100° C in HCl solution at pH 3.4. After the heating period, the solution was neutralized and applied to sensitivity discs as described above.

**Identification of compounds.** The pigments were identified by paper chromatographic means *(3, 9)*. The presence or absence of phloroglucinol and p-hydroxybenzoic acid *(15, 28)* in the heated solutions was determined by paper chromatography using 3 solvents: 1-butanol:acetic acid:water (4:1:5); 1-butanol:ethanol:borate buffer containing 15.8 g $Na_2BO_3$/liter (1:1:1); and benzene:acetic acid:water (20:5:saturated with water). The chromatograms were developed with diazotized sulfanilic acid soln *(1)*. Hydroxymethylfurfural and furfural *(12, 21, 35)* were tested for with the BAW solvent above, with 1-butanol:ethanol:water (45:5:50), and with phenol:water (80:20).

The infrared spectra of some of the anthocyanin compounds was determined using a Beckman IR 5 spectrophotometer. Both the mull method (using mineral oil) and deposition of the pigment on the NaCl prism were used. For the latter method, freshly distilled anhydrous ethanol was used as the solvent and the solvent was rapidly evaporated with a hair dryer.

### RESULTS

**Synthetic compounds.** Of the 5 synthetic compounds, 5-desoxy-3′-methoxy-apigenindin-chloride-4′-methyl-ether, apigeninidin-chloride, and 5-desoxy-apigeninidin-chloride-4′methyl-ether were inhibitory toward *S. aureus* but not toward *E. coli* nor *L. casei*. The other 2 compounds, 3′-methoxy-apigeninidin-chloride-4′-methyl-ether and apigeninidin-chloride-4′-methyl-ether, had no effect on any of the organisms. The degree of activity of the compounds toward *S. aureus* is listed in Table 1 and shown in Figures 1 and 2. Pelargonidin-3-monoglucoside extracted from strawberries exhibited activity toward the 3 organisms tested. It was generally inhibitory toward *E. coli* and *S. aureus*, but at the upper levels of application, evidence of both stimulation and inhibition could be seen around the sensitivity discs when *L. casei* was the test organism. The relationship of concentration to microbial activity is tabulated in Table 2 and shown in Figure 3.

Tests were conducted with pelargonidin prepared by hydrolyzing the pelargonidin-3-monoglucoside with acid and with an

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[c] Presented at the Twentieth Annual Meeting of the Institute of Food Technologists, May 18, 1960, San Francisco, California.

TABLE 1

**Effect of synthetic apigeninidin compounds on growth of Staphylococcus aureus**

| Compound | Inhibition[1] | |
|---|---|---|
| | 5 mg/disc | 10 mg/disc |
| 5-Desoxy-3′-Methoxy-Apigeninidin-Chloride-4′-Methyl-Ether | −8 | −12 |
| Apigeninidin-Chloride | −4 | −6 |
| 5-Desoxy-Apigeninidin-Chloride-4′-Methyl-Ether | −2 | −5 |
| 3′-Methoxy-Apigeninidin-Chloride-4′-Methyl-Ether | 0 | 0 |
| Apigeninidin-Chloride-4′-Methyl-Ether | 0 | 0 |

[1] Inhibitions = diameter (mm) of zone of inhibition minus diameter of disc.

TABLE 2

**Effect of concentration of pelargonidin-3-monoglucoside on growth of test organisms**

| Concn pigment mg/0.5″ disc | *E. coli* | | *L. casei* | | *S. aureus* | |
|---|---|---|---|---|---|---|
| | Stim | Inhb[1] | Stim | Inhb[1] | Stim | Inhb[1] |
| 1.0 | 0 | −1.8 | 0 | 0 | 0 | 0 |
| 2.0 | 0 | −3.3 | 0 | 0 | 0 | 0 |
| 3.0 | 0 | −3.8 | 0 | −1.0 | 0 | −1.0 |
| 6.0 | 0 | −9.5 | + | −2.8 | 0 | −7.3 |
| 12.0 | 0 | −13.3 | + | −8.3 | 0 | −13.3 |
| 15.0 | 0 | −18.3 | + | −12.8 | 0 | −18.8 |

0 = no measurable effect.
+ = stimulation.
[1] Values listed = diameter (mm) of zone of inhibition minus diameter of disc.

TABLE 3

**Action of fractions extracted from Pinot Noir wine**

| Fraction | Stimulation or inhibition[1] | | | | | |
|---|---|---|---|---|---|---|
| | *E. coli* | | *S. aureus* | | *L. casei* | |
| | 5 mg/disc | 10 mg/disc | 5 mg/disc | 10 mg/disc | 5 mg/disc | 10 mg/disc |
| $W_0$ | 4 | 6 | 19 | 25 | 11 | 24 |
| $W_1$ | −3 | −5 | 11 | 25 | 7 | 24 |
| $W_2$ | 3 | 6 | 22 | 27 | 13 | 17 |
| $W_3$ | 8 | 14 | 7 | 10 | 5 | 10 |
| $W_4$ | 13 | 18 | 0 | 0 | 0 | 0 |
| $W_5$ | 10 | 18 | 10 | 13 | 5 | 3 |
| | | | | | −5[2] | −3[2] |
| $W_6$ | 7 | 15 | 7 | 15 | 6 | 12 |

[1] Inhibition or stimulation shown as diameter (mm) of zone minus diameter of disc. Negative values denote inhibition.
[2] Zone of inhibition immediately surrounded by zone of stimulation.

anthocyanase preparation at pH 3.2 *(13, 37)*, but no action on the organisms could be discerned by the sensitivity disc method. Because of the low solubility of the aglucone, not only was the pigment added to the sensitivity disc but it was also added directly to the agar by depositing 10 mg in 0.05 ml of a 95% ethanol solution. Preliminary study established that deposition of 0.05 ml of 95% alcohol did not affect growth. As a double check, control drops of alcohol containing no pigment were added to the same plates as drops containing the pigment. Neither the alcohol alone nor the pigment dissolved in it affected growth.

Pigments extracted from Pinot Noir wine also varied in activity. Some fractions stimulated growth of some organisms while inhibiting a different organism and, as with the pelargonidin-3-monoglucoside, some fractions both inhibited and stimulated growth of the same organism. Fraction W-1, delphinidin-3-monoglucoside, was the only anthocyanin which inhibited growth of *E. coli*. *L. casei* either was not acted upon or it was stimulated by the various fractions. In general, *S. aureus* was stimulated but pigment W-5 (not identified) contained both stimulating and inhibiting factors. Intact wine, adjusted to pH 6.8, did not stimulate growth.

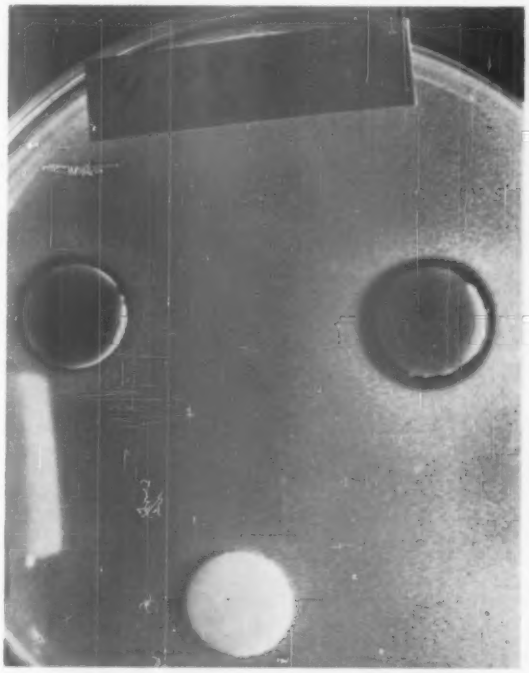

**Figure 1. Influence of 5 mg and 10 mg apigeninidin-chloride on growth of S. aureus. Disc containing 5 mg, upper left; disc containing 10 mg, upper right; control disc at bottom.**

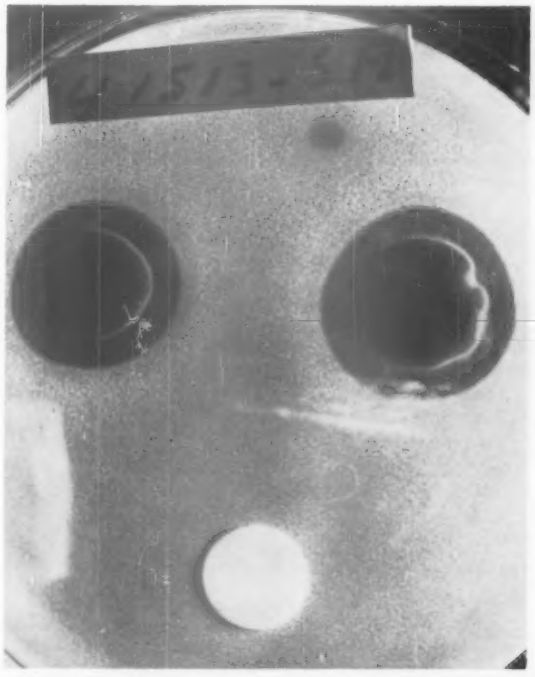

**Figure 2. Influence of 5 mg and 10 mg 5-desoxy-3′-methoxy-apigeninidin-chloride-4′-methyl-ether on growth of S. aureus. Disc at upper left contains 5 mg; at upper right, contains 10 mg. Control disc at bottom.**

**TABLE 4**

**Action of fractions extracted from grape juice**

| Fraction | Hours incub | Stimulation or inhibition[1] | | | | |
|---|---|---|---|---|---|---|
| | | *E. coli* | | *L. casei* | | *S. aureus* |
| | | 5 mg/disc | 10 mg/disc | 5 mg/disc | 10 mg/disc | 5 mg/disc |
| $G_2$ | 24 | 5 | −11 | −8 | −15 | −10 |
| | 48 | 5 | −12 | .... | −7 | 12 |
| | | | | | 15 | |
| $G_3$ | 24 | .... | 0[2] | 0[2] | 0[2] | −4 |
| | 48 | .... | −3 | .... | 20 | 10 |
| $G_4$ | 24 | 0 | 0 | 13 | 20 | 16 |
| | 48 | 0 | 0 | .... | 21 | .... |

[1] Diameter of zone minus diameter of disc in millimeters. Negative values denote inhibition.
[2] Slight inhibition.

Although Fraction W-2 isolated from Pinot Noir wine stimulated growth of the 3 organisms, Fraction G-2, which had similar $R_f$ values but was extracted from Concord-type grape juice, was not stimulatory toward all the organisms. At a level of 10 mg per disc, Fraction G-2 inhibited *E. coli* and it showed both types of activity toward *L. casei*. Malvidin-3-monoglucoside (Fraction G-4) was either stimulatory or without activity toward the test organisms. The activities observed are listed in Tables 3 and 4. It may be noted from Table 3 that Fraction W-4 (malvidin, the aglucone) stimulated *E. coli* and was without effect on the other two organisms. It may also be noted from Table 4 that inhibition at 24 hr was sometimes transitory and that stimulation of growth was evident by the end of 48 hr incubation.

**Identity of compounds.** The $R_f$ values of the different compounds in different solvents are shown in Tables 5, 6, and 7. The $R_f$ values for apigeninidin-chloride did not agree with those reported for the natural apigeninidin compound *(9)*. The pigments, however, did conform to theories concerning chromatographic movement as influenced by structural configuration *(3, 10, 33)*.

Where identification of natural pigments could be made with reasonable certainty, the identity of the pigment is given in Tables 6 or 7. Some of the compounds listed are suspected not to be anthocyanin pigments judging by their lack of color or other characteristics, but the $R_f$ values are listed to indicate the presence of these compounds.

**Action of heated pigments.** Many foods which contain anthocyanin pigments are heated at some stage during their preparation or processing. The various pigments therefore were heated for different lengths of time to learn the extent to which heating affects the microbial properties of the pigments. (It is recognized, of course, that both the grape juice and the wine had already received some heating during processing.) The results of the heating studies are listed in Tables 8, 9, and 10. In general, it may be noted from Table 8 that apigeninidin-chloride and 5-desoxy-apigeninidin-chloride-4′-methyl-ether lost their ability to inhibit the organisms at some time during the heating period but that activity was regained, and that 3′-methoxy-apigeninidin-chloride-4′-methyl-ether, which originally did not inhibit *S. aureus*, affected growth after 90 and 120 minutes' heating. Against *L. casei*, none of the synthetic compounds were originally inhibitory, but 5-desoxy-apigeninidin-chloride-4′-methyl-ether and 3′-methoxy-apigeninidin-chloride-4′-methyl-ether became inhibitory after 60 minutes heating. No inhibition could be demonstrated after 120 min heating (Table 9).

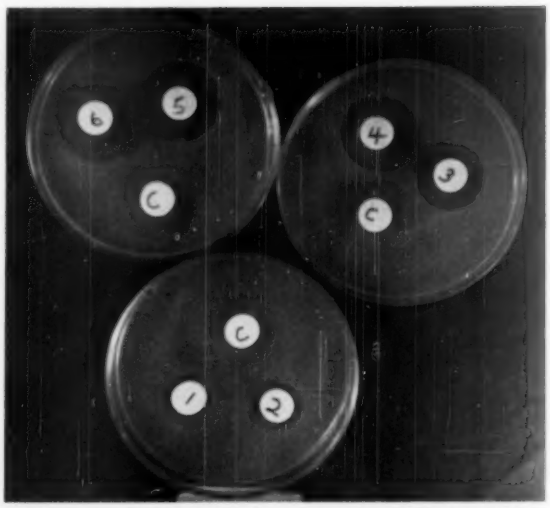


**Figure 3. Relationship of concentration of pelargonidin-3-monoglucoside to inhibition of E. coli. The respective concentration of pigment for discs 1, 2, 3, 4, 5, and 6 were, respectively, 1.0, 2.0, 3.0, 6.0, 12.0, and 15.0 mg. Discs labeled "C" are control discs.**

Paper chromatographic studies established that phloroglucinol was formed when apigeninidin-chloride, apigeninidin-chloride-4′-methyl-ether, and 3′-methoxy-apigeninidin-chloride-4′-methyl-ether were heated.

From Table 10, it may be noted that if heating affected the wine fractions at all, the effect was to make them stimulatory.

**Infrared analysis.** Infrared spectral analysis was made of some of the compounds in an effort to learn whether infrared analysis could be of aid in supplementing paper chromatography as a means of identifying compounds. The infrared curves for 3 grape fractions and for the 5 synthetic compounds are shown in Figures 4 and 5. Although fraction G-10 was not considered to be an anthocyanin by paper chromatography, its curve was similar to that of G-2 which was an anthocyanin and to G-6 which is considered to be an anthocyanin although not identified. The 2 curves for pelargonidin-3-monoglucoside represent a freshly extracted and purified compound and one which had been stored for several months. The absorption points of the grape pigments and pelargonidin-3-monoglucoside

**TABLE 5**

**$R_f$ values for apigeninidin compounds**

| Fractions | $R_f$ values[1] | | | | | Description | |
|---|---|---|---|---|---|---|---|
| | BAW | 1% HCl | HAc-HCl | Forestal | Formic | Visible light | U.V. light |
| Apigeninidin-Chloride | 0.90 | 0.32 | 0.43 | 1.00 | 0.81 | Orange pink | Pink |
| Apigeninidin-Chloride-4′-Methyl-Ether | 0.93 | 0.42 | 0.47 | 1.00 | 0.88 | Orange pink | Pink |
| 5-Desoxy-Apigeninidin-Chloride-4′-Methyl-Ether | 0.92 | 0.18 | 0.20 | 0.70 | | Yellow | Yellow-green |
| | | | 0.78 | 1.00 | 1.00 | Yellow | Yellow-green (fluorescence) |
| 3′-Methoxy-Apigeninidin-Chloride-4′-Methyl-Ether | 0.77 | 0.03 | 0.46 | 1.00 | 0.92 | Salmon pink | Red |
| 5-Desoxy-3′-Methoxy-Apigeninidin-Chloride-4′-Methyl-Ether | 0.84 | 0.19 | 0.28 | 0.68 | | Orange | Yellow-brown |
| | | | 0.81[2] | 1.00[2] | 0.97 | Orange | Yellow-brown (fluorescence) |

[1] Solvent ratios: n-butanol:acetic acid:water (4:1:5); 1% hydrochloric acid in water; acetic acid: hydrochloric acid:water (82:15:3); Forestal (acetic acid: hydrochloric acid:water) (30:3:10); formic acid: hydrochloric acid:water (5:2:3).
[2] Major fraction.

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TABLE 6

$R_f$ values and tentative identification of isolated wine compounds

| | $R_f$ | | | Description | | Tentative identification |
|---|---|---|---|---|---|---|
| | BAW | Forestal | 1% HCl | Visible light | U.V. light | |
| $W_4$ | ..... | ..... | ..... | Grey | ..... | ..... |
| $W_1$ | 0.13 | 0.67 | 0.04 | Purple | ..... | Delphinidin-3-monoglucoside |
| $W_3$ | 0.18 | 0.46 | 0.17 | Lavender pink | ..... | ..... |
| $W_2$ | 0.28 | 0.63 | 0.09 | Salmon pink | ..... | Delphinidin |
| $W_5$ | 0.50 | 0.68 | 0.05 | Strawberry pink | Fluorescence | Malvidin |
| $W_6$ | 0.64 | 0.65 | 0.26 | ..... | White fluor | ..... |
| $W_7$ | 0.76 | 0.70 | 0.42 | ..... | White fluor | ..... |

were checked against the absorption spectra of various glycosides of cyanidin as determined by Farmer *(7)* and very kindly furnished to us by him. Phillips determined the infrared spectra of antirrhinin, chrysanthemin, and pelargonidin for Li and Wagenknecht *(19)*. The values for antirrhinin were supplied by Li *(18)*. There were distinct similarities between Farmer's curves *(7)* and ours and also points of disagreement which may be related to the structural differences of the compounds. Some of the absorption points furnished by Li *(18)* likewise were identical or close to those observed in this study. The infrared curves for the synthetic compounds were, as is evident, different from the natural compounds. The synthetic and natural compounds differ not only in glycosidation but also in other structural groupings.

## DISCUSSION

This study and the previous one *(27)* are alike in that evidence of stimulation of growth by natural anthocyanin pigments was obtained. In the previous investigation, a decrease in the lag phase of growth was noted when growth rate studies in liquid media were conducted. In this study, accelerated multiplication was noted as a dense ring surrounding the sensitivity discs.

One possibility for the stimulation observed is that the pigments were not pure and that the effect observed may merely represent stimulation due to contaminating non-glycosidic sugar. Although there might have been traces of sugar adsorbed onto the crystallized glycosides, paper chromatography did not demonstrate such. Furthermore, the medium used contained 0.1% dextrose plus a small amount of additional sugar derived from the beef extract contained in the TGY agar. Even if traces of non-glycosidic sugar were present, the amount would be extremely small compared with that already present in the medium since no more than 10 mg of pigment was added to the sensitivity disc and all the pigment did not migrate from the disc. It appears likely that if stimulation resulted from the presence of sugar in the glycoside, the stimulation arose because the organisms had the ability to break the glycosyl bond, deriving energy thereby. It is also possible that in the re-establishment of equilibrium between $\alpha$ and $\beta$ glucose *(2)* that the organism can use one form more effectively than the other.

The fact that some stimulation of *S. aureus* resulted when the 3′-methoxy-apigeninidin-chloride-4′-methyl-ether was heated 90 and 120 min suggests that stimulation represents a mode of action other than breaking of the glycosyl bond. Schauffstatter *(34)* also observed that flavone compounds affected the growth of bacteria. One way in which compounds might either stimulate or inhibit the organisms would be through poising of the redox potential at an especially favorable or unfavorable level. Either the sugar or the anthocyanin might do this *(30, 32)*. Another means whereby the pigments might act is through chelation of essential nutrients. Kelley and Watts *(16)* have demonstrated that cyanidin chelates copper readily. Chelation by flavonoid compounds has been studied by Clark and Geissman *(5)*.

One respect in which the present study may disagree with prior work concerns wine pigment No. W-4 which stimulated growth and was identified as malvidin. Jensen *(14)* found that a partially-demethoxylated malvidin was bactericidal. The difference in degree of methoxylation might account for the discrepancy in results. This study agrees with that of Masquelier *(22)* who observed that pelargonidin was without bactericidal activity.

The $R_f$ values of the 4 pigmented compounds extracted from the Pinot Noir wine agreed closely with

TABLE 7

$R_f$ values and tentative identification of isolated grape compounds

| | $R_f$ | | | Description | | Tentative identification |
|---|---|---|---|---|---|---|
| | BAW | Forestal | 1% HCl | Visible light | U.V. light | |
| $G_6$ | ..... | ..... | ..... | Blue | ..... | ..... |
| $G_1$ | 0.13 | ..... | ..... | Purple | ..... | Delphinidin-3-monoglucoside |
| $G_2$ | 0.19 | 0.44 | 0.18 | Light rose | Red fluor | Malvidin-3,5-diglucoside |
| $G_3$ | 0.33 | 0.64 | 0.05 | Pink red | Violet fluor | |
| $G_4$ | 0.37 | 0.81 | 0.07 | Light pink | Red fluor | Malvidin-3-monoglucoside |
| $G_5$ | 0.42 | 0.84 | 0.08 | Pink | ..... | ..... |
| $G_8$ | 0.47 | 0.83 | 0.03 | Light pink | ..... | ..... |
| $G_7$ | 0.58 | 0.80 | 0.04 | Light pink | ..... | ..... |
| $G_9$ | 0.66 | 0.73 | 0.05 | Pink | ..... | ..... |
| $G_{10}$ | 0.69 | 0.92 | 0.07 | ..... | Blue | ..... |

TABLE 8

**Effect of synthetic apigeninidin compounds after heating 30, 60, 90, and 120 minutes at 100° C on S. aureus**

| Compound | Heating periods and inhibition[1] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 30 | | 60 | | 90 | | 120 | |
| | 5 mg | 10 mg | 5 mg | 10 mg | 5 mg | 10 mg | 5 mg | 10 mg | 5 mg | 10 mg |
| Apigeninidin-Chloride | −4 | −6 | −1 | −3 | 0 | 0 | −1 | −2 | −1 | −3 |
| 5-Desoxy-Apigeninidin-Chloride-4′-Methyl-Ether | −2 | −5 | 0 | 0 | −2 | −4 | −1 | −3 | −1 | −4 |
| 3′-Methoxy-Apigeninidin-Chloride-4′-Methyl-Ether | 0 | 0 | 0 | 0 | −1 | −3 | ±1[2] | −3 ±2 | 2 | −2 ±2 |
| Apigeninidin-Chloride-4′-Methyl-Ether | 0 | 0 | 0 | 0 | 0 | −6 | 0 | 0 | 0 | 0 |

[1] Inhibition or stimulation zone in millimeters minus diameter (mm) of disc. Negative values denote inhibition.
[2] Also showed slight inhibition.

TABLE 9

**Effect of synthetic apigeninidin compounds after heating 30, 60, 90, and 120 minutes at 100° C on L. casei**

| Compound | Heating periods and inhibition[1] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 30 | | 60 | | 90 | | 120 | |
| | 5 mg | 10 mg | 5 mg | 10 mg | 5 mg | 10 mg | 5 mg | 10 mg | 5 mg | 10 mg |
| 5-Desoxy-Apigeninidin-Chloride-4′-Methyl-Ether | 0 | 0 | 0 | 0 | −4 | −7 | −2 | −4 | 0 | 0 |
| 3′-Methoxy-Apigeninidin-Chloride-4′-Methyl-Ether | 0 | 0 | 0 | 0 | −2 | −5 | −2 | −6 | 0 | 0 |

[1] Inhibition or stimulation zone in millimeters minus diameter (mm) of disc. Negative values denote inhibition.

those reported by Rankine, Kepner, and Webb *(29)* who compared the pigments of several varieties of grapes. Three of the pigments could be identified with a fair degree of certainty, but the identity of Fraction W-2 is in doubt. This fraction had the same $R_f$ values as Fraction G-2 which was identified as malvidin-3-5-diglucoside on the basis of $R_f$ values, fluorescence under ultraviolet light, and color in visible light. In spite of the almost complete identity of $R_f$ values, Fraction W-2 appeared to be different from Fraction G-2. The wine pigment did not fluoresce in ultraviolet light which suggests that it was not glycosylated at the fifth carbon position, and, from a biological point of view, the action of Fraction W-2 toward the 3 organisms was different in some respects from that of Fraction G-2. Ribereau-Gaynon *(31)* states that diglucosides are not found in *Vitis vinifera* grapes but that they are present in many other species. Fraction W-2 was similar in some respects to Fraction G-2, identified as malvidin-3-5-diglucoside, but it probably was not identical with it.

Identification of phloroglucinol as a breakdown product of anthocyanin pigment when heated in acid solution has not apparently previously been reported. In earlier studies in this laboratory, Pratt *(26)* identified phloroglucinol and p-hydroxybenzoic acid as intermediate or end products when pelargonidin-3-monoglucoside was heated for periods up to 2 hr at 180° F. Phloroglucinol was identified in this study when the synthetic compounds were heated. Michener, Thompson, and Lewis *(25)* have studied the influence of phlorglucin (phloroglucinol) on growth of a strain of the *Clostridia*, P. A. 3679. Walker and Link *(36)* have shown phloroglucinol to be toxic toward some organisms.

TABLE 10

**Action of pigments extracted from wine after heating for 30 and 60 minutes**

| Pigment | Time of heating min | Stimulation[1] | | | | | |
|---|---|---|---|---|---|---|---|
| | | *E. coli* | | *L. casei* | | *S. aureus* | |
| | | 5 mg | 10 mg | 5 mg | 10 mg | 5 mg | 10 mg |
| $W_0$ | 30 | 0 | 0 | 19 | 11 | 17 | 11 |
| | 60 | 10 | 6 | 18 | 12 | 10 | 7 |
| $W_1$ | 30 | 8 | 6 | 22 | 16 | 21 | 19 |
| | 60 | 0 | 0 | 22 | 17 | 26 | 19 |
| $W_2$ | 30 | 12 | 7 | 20 | 15 | 17 | 12 |
| | 60 | 13 | 9 | 15 | 12 | 16 | 12 |
| $W_3$ | 30 | 18 | 11 | 20 | 14 | 10 | 5 |
| | 60 | 17 | 10 | 0 | 0 | 11 | 8 |
| $W_4$ | 30 | 20 | 16 | 0 | 0 | 0 | 0 |
| | 60 | 19 | 15 | 0 | 0 | 0 | 0 |
| $W_5$ | 30 | 17 | 12 | 0 | 0 | 0 | 0 |
| | 60 | 15 | 10 | 0 | 0 | 0 | 0 |

[1] Diameter of zone minus diameter of disc in millimeters.

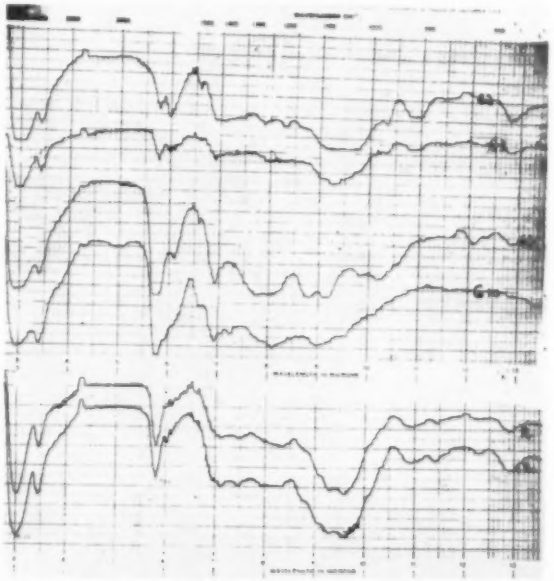

**Figure 4. Infrared spectral curves, grape and strawberry pigments. Curve G-2 is for malvidin-3, 5-diglucoside; G-3, G-6, and G-10, not identified. Curve Po is for a sample of pelargonidin-3-monoglucoside stored several months; curve Pn for a freshly-extracted and purified sample of pelargonidin-3-monoglucoside.**

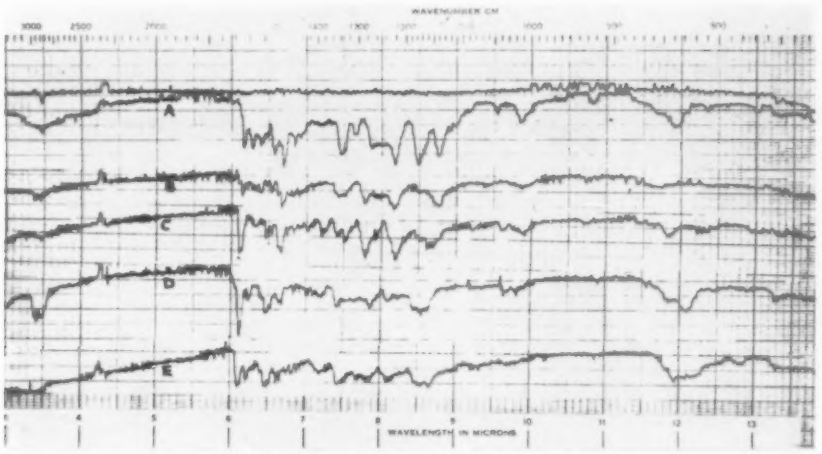


Figure 5. Infrared spectra of synthetic compounds. Curves A, B, C, D, and E, respectively, are for 5-desoxy-apigeninidin-chloride-4′-methyl-ether; 5-desoxy-3′-methoxy-apigeninidin-chloride-4′-methyl-ether; 3′-methoxy-apigeninidin-chloride-4′-methyl-ether; apigeninidin-chloride-4′-methyl-ether; and apigeninidin-chloride.


## SUMMARY

Pelargonidin-3-monoglucoside and delphinidin-3-monoglucoside inhibited the growth of *E. coli*. Malvidin-3,5-diglucoside stimulated growth at some stages and inhibited it at others. Delphinidin and malvidin stimulated growth.

The compounds, 5-desoxy-3′-methoxy-apigeninidin-chloride-4′-methyl-ether, apigeninidin-chloride, 5-desoxy-apigeninidin-chloride-4′-methyl-ether, and pelargonidin-3-monoglucoside, each inhibited the growth of *S. aureus*. Delphinidin-3-monoglucoside, malvidin-3,5-diglucoside, malvidin-3-monoglucoside, and delphinidin accelerated multiplication as evidenced by increased growth around the sensitivity discs.

*L. casei* generally was stimulated by malvidin-3,5-diglucoside and malvidin. At a concentration of 10 mg pigment per disc, malvidin-3,5-diglucoside inhibited growth, and pelargonidin-3-monoglucoside likewise was inhibitory.

Heating of synthetic compounds resulted in the loss of activity in some cases and the gaining of activity in other cases where the non-heated compound had been without activity.

The $R_f$ values of the compounds and the infrared spectra of some of the compounds were determined.

Liberation of phloroglucinol in acid solution during the heating of the synthetic compounds was demonstrated by paper chromatographic analysis.



### Acknowledgment

Appreciation is expressed to Hoffmann-LaRoche Inc., Nutley, New Jersey, for donation of the apigeninidin compounds used in the study.


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# Effect of Chlorine on Spores of *Bacillus coagulans*[a]

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(Manuscript received May 31, 1960)

The protection afforded by chlorination of drinking water to prevent the spread of disease bacteria is well known. These microorganisms are, for the most part, non-spore formers, easily killed by low concentrations of chlorine at room temperature. Comprehensive studies performed on spore-forming bacteria important in the spoilage of canned foods are lacking in the literature. Since the length of a process for canned foods is dependent upon the number of bacterial spores in the raw product at the time of the heat treatment, the bacterial "spore load" is of primary importance *(1)*. Process times are based on a "normal load" and contamination above this level might lead to spoilage; therefore, any decrease in the "spore-load" prior to heat treatment would be beneficial.

In-plant chlorination studies have been conducted by Somers *(13)* who found that chlorination reduced the total bacterial count. This study indicated that no increased corrosion of the equipment was noted where 5 ppm residual chlorine was maintained during operations, and 20 ppm for clean up periods. One of the benefits derived from in-plant chlorination, as proposed by Mercer and Somers *(11)* is killing of the vegetative cells of the organism which form spores, prior to spore formation.

In the tomato juice processing plant, the chopper, preheater, extractors, pumps, pipe lines and filler bowls cannot be chlorinated during normal operations and therefore, these points in the process lines have been listed as sources of contamination *(2, 14)*. The development of information needed for the sporicidal

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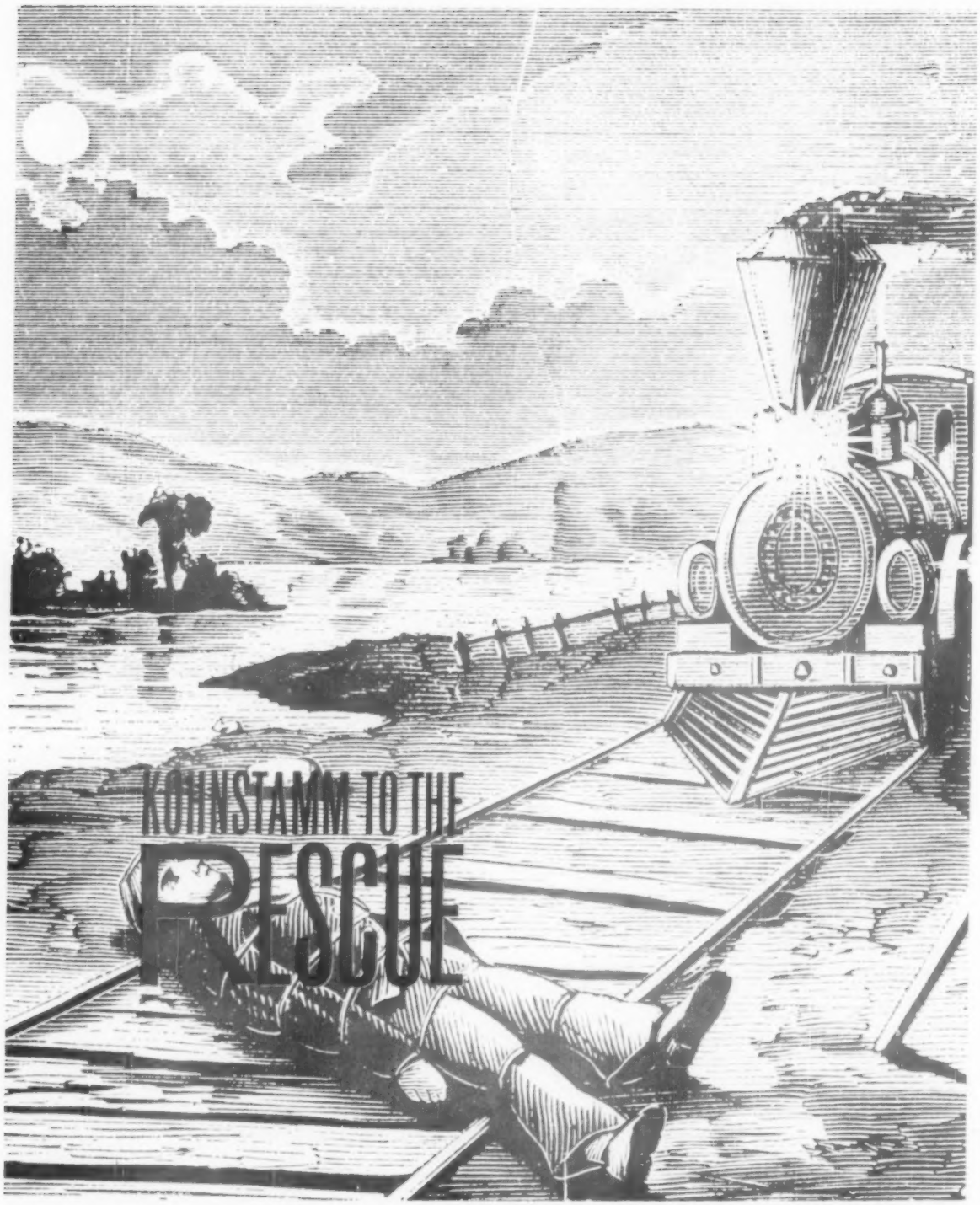


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effect of chlorine on the spores of this specific organism is desirable in order that the maximum kill of the spores may be accomplished during the clean up period.

This study was conducted to determine the resistance of *Bacillus coagulans* spores under different temperatures, chlorine concentrations and pH levels to correspond with possible food plant operative conditions.

## MATERIALS AND METHODS

The desirable chlorine concentrations for in-plant chlorination are 4 to 7 ppm during normal operations and 10 to 20 ppm during plant clean up *(11)*. Therefore, 5 10, and 20 ppm chlorine were selected for this study. The temperatures used in this investigation were 15, 20, 30, and 60° C, which represent the typical range of clean-up water conditions *(5)*.

The pH levels chosen for this study were 7.8, 6.8, and 4.5. The upper pH level is representative of Indiana well water *(8)* while the lower pH ranges would be expected to affect greater sporicidal action as reported by Brazis *et al (1)* and Levine and Rudolph *(10)*. The 4 different temperatures and the 3 pH levels were studied as variables at each of the chlorine concentrations. Thus, there were 12 experiments at each chlorine level, or a total of 36 experiments. Each experiment was replicated 3 times.

**Organism.** *Bacillus coagulans (B. thermoacidurans)* strain NRRL B-1175 was obtained from the culture collection maintained by the NURDD (Northern Utilization Research and Development Division), Peoria, Illinois.

*Preparation of spores.* Two ml from a stock ($1 \times 10^6$ spores per ml) spore suspension which had previously been heat shocked at 80° C for 10 min were transferred into three 250-ml Erlenmeyer flasks each containing 160 ml of thermoacidurans broth which was prepared from the various ingredients at the time of use. The pH was adjusted to 5.0 with HCl. The inoculated flasks were incubated at 45° C for 48 hr. The abundant vegetative growth from the broth was poured in a thin layer over the surface of thermoacidurans agar (Difco) in Roux flasks and incubated at 45° C for 2 weeks. Spores were then harvested by gently scraping the agar surface with sterile glass rods and using sterile water to wash off the scrapings. Scrapings were transferred to a sterile centrifuge bottle containing glass beads and shaken for 3 min. The harvested suspension was then centrifuged for 10 min at 1500 rpm to separate the spores from vegetative cells. Further, the suspension was washed in this manner 4 times with sterile distilled water followed by a wash in M/15 sterile, neutral phosphate buffer. The washed spores were then re-suspended in sterile phosphate buffer, heat shocked at 80° C for 10 min, and counted using standard plate-count technique on thermoacidurans agar (Difco). After the spore concentration was determined, the spore suspension was adjusted to $1 \times 10^6$ spores per ml and 10 ml amounts dispensed into screw capped sterile tubes containing glass beads. The tubes were then stored at 4–6° C in a refrigerator.

**Preparation of chlorine solutions.** A stock chlorine solution of approximately 200 ppm concentration was prepared from a 5.25% sodium hypochlorite solution. Aliquots were then transferred from the stock chlorine solution to sterile M/15 buffer, which was previously adjusted to the appropriate test pH to give final concentrations of 5, 10, or 20 ppm chlorine. The initial concentration of chlorine was then determined for each treatment by the starch iodide test *(12)*.

**Chlorine neutralization.** A 0.5% sodium thiosulfate solution was used for neutralizing the germicidal action of chlorine following each sampling interval of the sporicidal test *(9)*. No residual chlorine was detectable following neutralization treatments. The neutralizer was shown to be non-toxic under the conditions of this experiment *(9)*.

**Test procedure.** The test was modified slightly from the method of Chambers *(3)*. Test water at the appropriate pH level and chlorine concentration was measured in 99-ml amounts into 3 sterile cotton plugged 250-ml Erlenmeyer flasks and placed in a water bath at the definite test temperature. A 99-ml control flask was also placed into the water bath to be used in determining the initial chlorine concentration at the beginning of the test period.

Following temperature adjustments in the flasks, one ml of the heat-activated spore suspension was added and thoroughly mixed with the contents of each of the 3 treatment flasks. The initial chlorine concentration was then determined, and reported as the residual chlorine concentration. At the designated time intervals, an aliquot was transferred from the treatment flasks to the neutralizer blanks. Further dilutions were made into neutral phosphate buffer blanks and plated in triplicate. The control, containing no chlorine, was likewise prepared and plated in triplicate. Additional controls determined at the end of the test period included final residual chlorine and pH values of the test treatments. All plates were incubated at 52° C for 48 hr and counted.

## RESULTS AND DISCUSSION

In an earlier report, Mercer and Somers *(11)* concluded that inplant chlorination conditions were not practical in reducing the number of bacterial spores. However, the influence of various temperatures was apparently not studied in connection with germicidal action on spores. In addition, they reported that the type of chlorine used was important in high temperature studies as gaseous chlorine is less soluble under these conditions. Hadfield *(6)* reported no chlorine loss at 55° C during a 3-hr period. Therefore, he suggested the practice of high temperature chlorination in cleaning of food processing equipment. Sodium hypochlorite solution was used in this study. The maximum chlorine lost as shown in Table 1 occurred at 60° C; however, this was considered slight from a practical viewpoint.

TABLE 1

Chlorine lost during experiments at 60° C

| pH | Ave ppm free chlorine after exposure (time in minutes) | | | | Chlorine lost during experiment in ppm |
|---|---|---|---|---|---|
| | 0 | 10 | 30 | 60 | |
| 4.5 | 4.9 | | | 3.3 | 1.6 |
| 6.8 | 5.1 | | | 3.5 | 1.6 |
| 7.8 | 4.8 | | | 3.2 | 1.6 |
| 4.5 | 10.0 | | 8.6 | | 1.4 |
| 6.8 | 9.91 | | | 7.4 | 2.5 |
| 7.8 | 9.0 | | 7.6 | | 2.6 |
| 4.5 | 21.3 | 19.0 | | | 2.3 |
| 6.8 | 20.1 | 17.8 | | | 2.3 |
| 7.8 | 19.0 | 15.0 | | | 4.0 |

Considering the sporicidal effect at various pH levels, with temperature and chlorine constant, the sporicidal action increases with decreasing pH values. For example, when the temperature and chlorine were held at 15° C and 20 ppm, respectively, the times to kill 90% of the spores at pH values of 7.8, 6.8, and 4.5 were 13.0, 9.0, and 4.0 min, respectively, as shown in Figures 1, 2, and 3. At 60° C and 20 ppm chlorine, the times to kill 90% of the spores were 1.0, 0.25, and 0.30 min at the same pH levels. These findings agree with Brazis *et al (1)* and Levine and Rudolph *(10)*.

Other factors constant, the results indicated that the sporicidal action increased sharply with increasing

temperatures as shown in Figures 1, 2, and 3. At pH 7.8 and 20 ppm chlorine, the times to kill 90% of the spores at 15, 20, 30, and 60° C were 13, 9, 5, and 1 min, respectively.

A practical time to bring about a 90% kill can also be achieved by increasing the chlorine content. Unless long periods of contact are expected in waters at 15° C, the chlorine content would have to be used at 20 ppm. For example, at pH 7.8 and 15° C, the time to kill 90% of the spores with approximately 5 ppm chlorine was 75 min.

Although the problem of corrosion of chlorine on stainless steel under the conditions of this experiment were not determined, it was felt that such short contact times would not cause corrosion since contact times of 200 ppm of liquid sodium hypochlorite have been recommended for sterilizing tubular heaters with contact times of 5 min *(7)*.

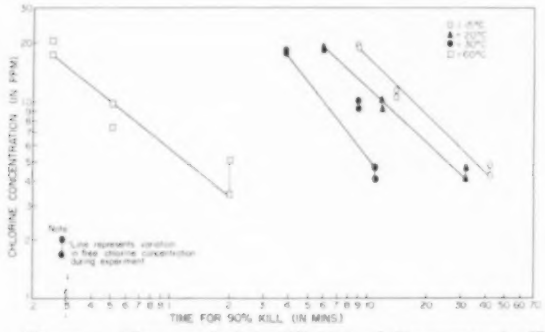


**Figure 1. Time required to kill 90% of the spores at pH 6.8.**

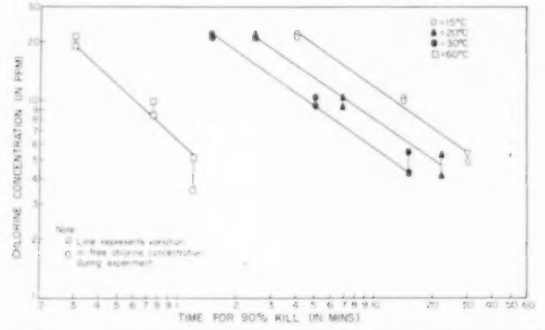


**Figure 2. Time required to kill 90% of the spores at pH 4.5.**

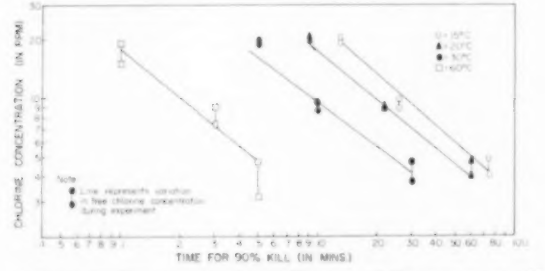


**Figure 3. Time required to kill 90% of the spores at pH 7.8.**

## SUMMARY

The sporicidal effect of chlorine on the spores of *Bacillus coagulans* was influenced by pH, temperature, and chlorine concentration. By increasing the temperature to 60° C (140° F), the sporicidal effect was in the range of practical use in a cannery. The amount of chlorine lost, when 60° C was used, was considered insignificant. The sporicidal effect was also increased to a practical point when the chlorine content was increased to 20 ppm.

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# Effect of Ripeness Level on Consistency of Canned Tomato Juice[a]

(Manuscript received May 18, 1960)

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Consistency of canned tomato juice varies with heat treatment of the fresh fruit during extraction *(8)*. Robinson *et al* studied the effect of manufacturing methods on consistency *(23)* and degree of settling in tomato juice *(24)*. Hand *et al (6)* reported that quantity, configuration, and characteristics of the suspended particles influence juice consistency. Variations in consistency obtained by using different finisher speeds were explained through microscopic examinations of the juice. When the finisher was run at a low speed, a relatively small amount of particles was incorporated into the juice, most of which were spherical, and the juice obtained was of low viscosity. Conversely, when the finisher was run at a high speed, large quantities of particles were incorporated into the juice, most of which were elongated, and the juice was of high viscosity. Whittenberger and Nutting *(29)* studied the effect of tomato cell wall structure on the consistency of tomato juice. They found that juice obtained from the seed cavities only was devoid of cell walls and was of thin consistency. They also reported an increase in tomato juice consistency after washing the solids and making the juice up to its original volume with distilled water, which caused the cell walls to expand and become more hydrophilic. Luh, Leonard, and Dempsey *(14, 15)* reported that tomato pulp consistency depends on tomato variety and the quantity of pectic materials present in the puree. Davis *et al (3)* studied consistency of tomato puree with various viscosimeters.

Kertesz and McColloch *(9)* found that pectic substances in John Baer tomatoes did not change at any of the three stages of maturity. Appleman and Conrad *(1)* and Stier *et al (26)* studied the transformations which occur in fruit during during maturation, and reported that protopectin changes to soluble pectin during tomato ripening. Kassab *(7)* and Hall and Dennison *(5)* also found that ripening is accompanied by a decrease in protopectin and an increase in soluble pectin. Great variations in the proportion of total pectin substances have been found in tomatoes *(13)*; however, information is meager about the effect of ripeness level on tomato juice consistency.

In the present work, variations in pectin, cellulose, and consistency of tomato juices canned from fruits of different ripeness levels are reported. Various factors influencing tomato juice consistency are discussed.

## EXPERIMENTAL METHODS AND MATERIALS

**Tomatoes.** Improved Pearson tomatoes were direct seeded in fine, sandy-loam soil on April 21, 1958, in Davis, California. Ammonium sulfate fertilizer was side dressed at the rate of 100 lb of nitrogen per acre, and normal irrigation practices were followed except for an additional irrigation immediately after the first harvest. Harvests were made on September 8 and 23, and October 21. A representative sample of 40 tomatoes was taken from 200-lb lots of each ripeness class for analysis: (a) Pink (entirely pink, no green), (b) Firm or table-ripe (full red color, minimum eating ripeness), (c) Canning-ripe (intense red coloring, but still firm), (d) Soft-ripe (perceptible softening, but no decay, and (e) Field run (comparable to commercial pick). The fruit was stored overnight at 32° F and made into juice the following day.

**Canning of tomato juice.** Tomatoes in 200 lb lots were sorted according to the classification mentioned above, washed, stemmed, macerated in a spike pump, heated to 230° F for 10 sec in a wiped-film heat exchanger, and then pumped into a juice extractor with a 0.033 inch screen. The product was mixed with 0.60% cannery salt, heated in a steam-jacketed kettle to 190° F, filled into No 2½ cans with a head space of ⅜ to ½ inch, and sealed. The product was heated in rotary cooker at 212° F for 10 min, and cooled to 110° F in a rotary water cooler for 7 min.

### Analytical procedures

*Total and soluble solids.* Ten g of the sample was weighed into a tared aluminum dish. The sample was dried for 8 hr at 70° C under 27 inches of vacuum and a slow stream of dried air. Soluble solids were determined by reading the Brix scale on a Zeiss-Opton Refractometer. The readings were corrected to 20° C.

*pH.* The pH was measured with a Beckman glass electrode pH meter.

*Total titrable acidity.* Duplicate 10-g samples of tomato juice were weighed into 250 ml beakers and 200 ml of distilled water was added. The resulting mixture was titrated with 0.1 N sodium hydroxide until it reached a pH value of 8.0 on the Beckman pH meter. Results were reported as per cent citric acid.

*Objective color evaluation.* Forty tomatoes were used for each sample. The cross sectional areas of two tomato halves were graded for color by the Agtron E Colorimeter *(28)*, which measured the ratio of green (546 mme) to red (640 mme) reflectance. The average of 20 readings was reported. According to the California Department of Agriculture Standards, the Agtron E readings for tomato color grades are as follows: well-colored, 0 to 46; fairly well-colored, 47 to 67; and off-color, 68 or higher.

*Subjective color evaluation.* The samples were graded visually for skin color under a Macbeth standard light source. A panel of 7 persons scored 3 replications of the samples. An excellent sample was scored 9 to 10, good 7 to 8, fair 5 to 6, poor 3 to 4, and very poor 1 to 2.

*Consistency of tomato serum.* About 700 g of the tomato juice was centrifuged at 1800 rpm for 15 min to obtain the serum. Determinations of serum and juice consistency were made at 20° C with the Brookfield Synchro-lectric Viscometer

[a] Presented at the Twentieth Annual Meeeting of the Institute of Food Technologists, San Francisco, California, May 18, 1960.

[b] Present address: Instituto Tecnologico de Monterrey, Department de Quimica, Sucursal "J", Monterrey, Nuevo Leon, Mexico.

(Model MVF, with spindle No 1 at 20 rpm). When the readings were out of scale, spindle No 2 was used at speeds of 2, 4, and 10 rpm. A log-log plot of apparent viscosity versus spindle speed was obtained. From the log-log plot the reading at 20 rpm was obtained. A pipette was also used to determine consistency. A tube 21 cm long and 3.5 cm in outside diameter was fed with 170 ml of the sample. The time required for 78 ml of the sample at 20° C to flow through the tip of the pipette, a capillary tube 2 cm long and 1.85 mm in inside diameter, was determined.

*Total pectin.* Total pectin content of the fresh tomatoes was determined by a modification of the method published by McCready and McComb *(20)*. Fresh tomatoes weighing 500 g were sliced and added gradually to 1250 ml of boiling 95% ethyl alcohol in a 2 liter wide-mouth balloon flask. The mixture was kept boiling for 10 min with an electric heater. The contents were cooled in a water bath, then mixed in a Waring blender for 1 min. The mixture was filtered through a Whatman No 2 filter paper under suction. The alcohol insoluble solids (AIS) were washed with sufficient 75% ethyl alcohol to remove the sugars, and then with 50 ml of purified 95% ethyl alcohol to remove other interfering substances. The product was dried in an oven at 45° C under 27 inches of vacuum and a slow stream of dried air for 48 hr. Two g of the dried AIS was mixed with 500 ml of 0.5% versene (sodium salt of ethylenediaminetetraacetic acid), treated with 1 N sodium hydroxide to reach pH 11.5, allowed to sequester for 30 min at room temperature, and then adjusted to pH 5.0 with 1 N acetic acid. Two ml of filtered 10% Pectinol "O" solution was added, and the mixture was left overnight with 2 to 3 drops of toluene added as a preservative.

The sample was diluted to 500 ml and filtered through a Whatman No 1 filter paper. A 25-ml aliquot was taken and diluted with distilled water to a volume of 200 ml. Two ml of the sample was placed in a Pyrex test tube containing 12 ml of concentrated sulphuric acid. The sample was thoroughly mixed with the acid, cooled in an ice water bath for 15 min, and then heated in a boiling water bath for 10 min, counting the time when the water started to boil. The sample was cooled to room temperature, mixed thoroughly with 1.0 ml of 0.15% carbazole in purified ethyl alcohol, and allowed to stand for 25 min. The intensity of the pink color was measured in a Klett-Summerson photo-electric colorimeter with a green filter (No 54). A blank was run by the same procedure, and a standard curve constructed with known amounts of galacturonic acid.

*Protopectin.* Protopectin determination was made by modifying the methods described by McCready and McComb *(20)* and Postlmayr *et al (22)*. Preparation of the sample was the same as for total pectin determination, except the AIS was extracted with 150 ml of water in a continuous shaker for 16 hr. Samples were filtered through a Whatman No 1 filter paper with a layer of Super-cel and further extracted with 300 ml of water for 30 min in a continuous shaker. The protopectin was dried by suction and sequestered in 300 ml of 0.5% versene for 1 hr after the pH was adjusted to 11.5. The rest of the method is the same as the total pectin procedure described above.

*Galacturonic acid.* An ion exchange procedure was used with an Amberlite-IRA 400 anion exchanger in the carbonate form, as described by Borenstein *et al (2)*. This procedure made possible the quantitative separation of the acid from ethanolic extracts of tomatoes. The method was modified by using carbazole for color development instead of naphthoresorcinol *(19)*. The filtrate from the AIS determination was mixed with 100 ml of distilled water, treated with 1 N NaOH to pH 8.0, and passed through a 20 x 2 cm column which was packed with 24 g of prepared Amberlite IRA 400 in the carbonate form. The galacturonic acid was eluted from the column with 200 ml of 1 N $Na_2CO_3$ and the eluate was diluted with 1 N $Na_2CO_3$ to a volume of 250 ml.

Two ml of the eluate was used for the colorimetric determination by the carbazole method. The same procedure as that for total pectin determination was used.

*Cellulose.* A modification of the method described by Viles *et al (27)* was used. Ten ml of tomato juice was mixed with 70 ml of 80% ethanol in a 90 ml centrifuge tube. The mixture was centrifuged at 1800 rpm for 20 min. After decantation of the supernatant, the residue was washed 4 more times with 60 ml portions of hot 80% ethanol until a qualitative test with anthrone showed an absence of green color.

Pectin was extracted from the sugar-free residue with 100 ml of 0.5% versene, and the pH of the solution was adjusted to 7.0 with 0.1 N sodium hydroxide. The solution was transferred to a 250 ml Erlenmeyer flask, treated with a few drops of toluene as a preservative, kept at room temperature, and shaken for 21 hr. The solution was centrifuged and filtered through a sintered glass crucible of medium porosity. The pulp was washed several times with acetone to remove the soluble pigments. The product was dried, treated with cold 60% $H_2SO_4$, transferred to a volumetric flask, and diluted with 60% sulfuric acid to 50 ml in a volumetric flask. Ten ml of the anthrone reagent was mixed with 2 ml of the sample in a 25 x 30 Pyrex tube, heated for 12 min in a boiling water bath, and then cooled for 10 min in cool water. The absorbance of the green-colored solution was determined in a Klett-Summerson colorimeter with a No 66 red filter. A standard curve was constructed with 0, 12.5, 25, 50, 100, and 200 meg of cellulose solubilized in the manner described above.

## RESULTS

**Effects of ripeness level on color, pH, and acidity.** Ripeness level of the fresh tomatoes was evaluated by the Agtron Model E. The color of the cross sectional area of the tomato halves was measured as a ratio of green (546 mme) to red (640 mme) reflectance. When the tomatoes ripened, the increased red reflectance and decreased green reflectance caused a decrease in Agtron E readings. The relationship between Agtron E values and visual color score was shown to be linear (Figure 1). The correlation coefficient was −0.98. The regression line between the two variables is expressed by the equation:

$$Y = -0.107X + 12.48$$

where Y is the visual color score on a 1 to 10 scale, in which the best colored tomatoes were scored 10 points, and X is the average Agtron E reading. It appears that the Agtron E is useful as an objective criterion for measuring color of fresh tomatoes.

The pH, acidity, and Brix readings of juices canned at different ripeness levels are shown in Table 1. Ripening produced similar changes in pH and acidity among the early, middle, and late season juices. The pH value of the juice increased by 0.2 to 0.3 units as the fresh fruits ripened from the pink to soft-ripe stage. The titrable acidity decreased with ripening. Late season tomatoes were slightly higher in acidity than were the early and middle season fruits. In the majority of California canning tomatoes, the titrable acidity varied from 0.25 to 0.40%. Samples used in this investigation were slightly higher in acidity than usual, probably because of differences in soil

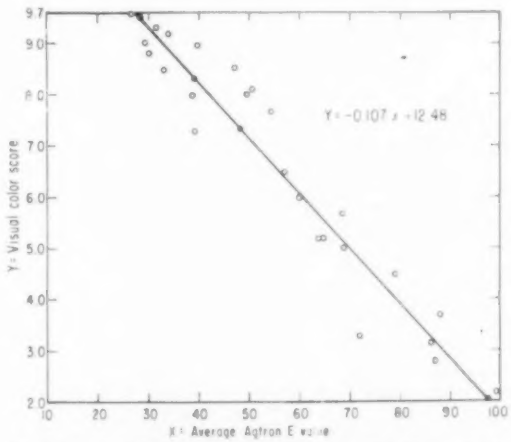


**Figure 1. Relationship between Agtron E value and visual color score of fresh tomatoes.**

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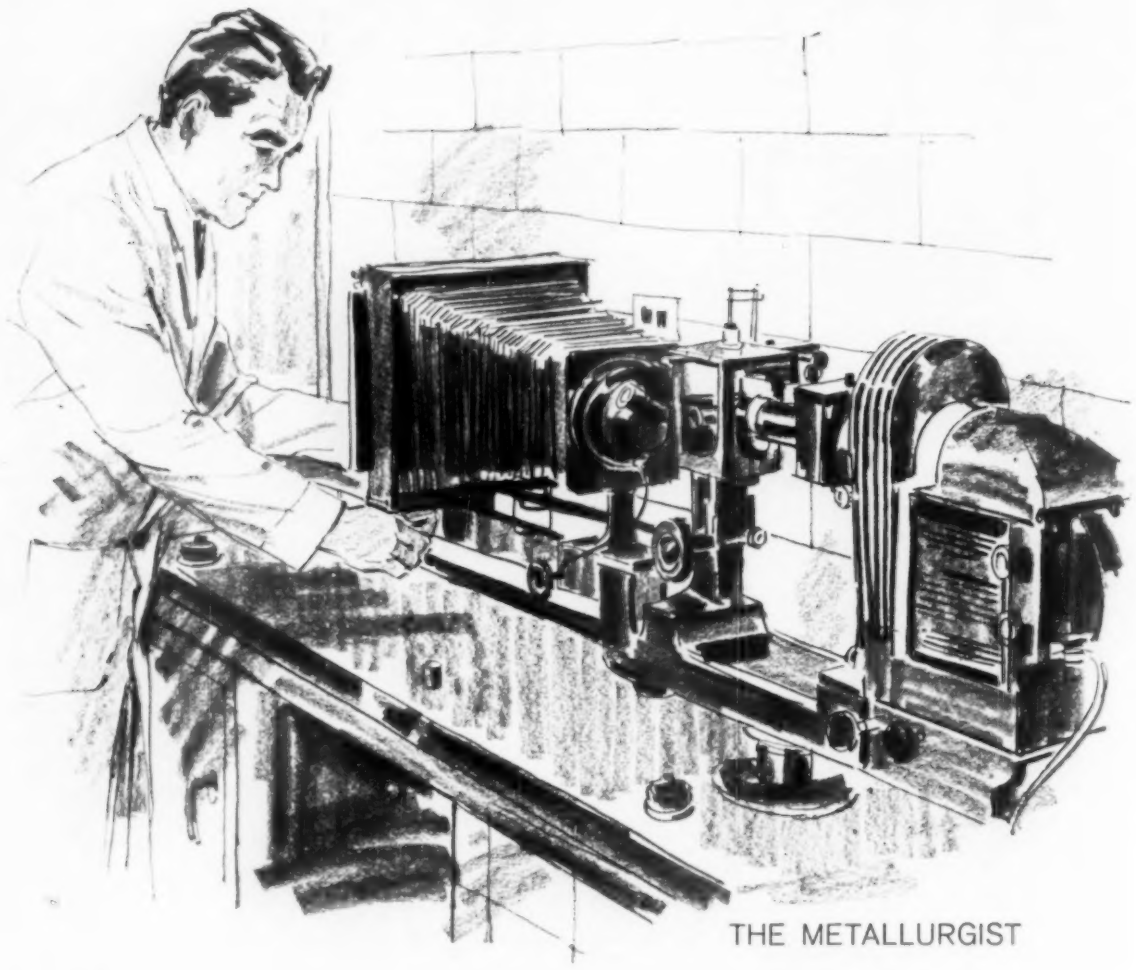

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**TABLE 1**

**Influence of ripeness level on chemical composition of canned tomato juice (improved Pearson variety, 1958 crop)**

| Sample | Ripeness level | Harvest date | Agtron E reading of fresh tomatoes[1] | pH | % Total acid as citric | Brix at 20° C |
|---|---|---|---|---|---|---|
| | Early Season | 9/8/58 | | | | |
| 1 | Pink | | 72 | 4.21 | 0.698 | 6.67 |
| 2 | Firm | | 64 | 4.32 | 0.661 | 6.87 |
| 3 | Canning-ripe | | 39 | 4.35 | 0.639 | 6.87 |
| 4 | Soft-ripe | | 32 | 4.40 | 0.547 | 6.67 |
| 5 | Field run | | 39 | 4.38 | 0.538 | 6.67 |
| | Middle Season | 9/22/58 | | | | |
| 6 | Pink | | 96 | 4.10 | 0.766 | 6.57 |
| 7 | Firm | | 69 | 4.12 | 0.724 | 6.77 |
| 8 | Canning-ripe | | 29 | 4.30 | 0.618 | 6.92 |
| 9 | Soft-ripe | | 27 | 4.40 | 0.569 | 6.67 |
| 10 | Field run | | 30 | 4.36 | 0.618 | 6.87 |
| | Late Season | 10/20/58 | | | | |
| 11 | Pink | | 79 | 4.11 | 0.869 | 6.32 |
| 12 | Firm | | 60 | 4.13 | 0.856 | 6.47 |
| 13 | Canning-ripe | | 33 | 4.28 | 0.714 | 6.57 |
| 14 | Soft-ripe | | 29 | 4.31 | 0.660 | 6.47 |
| 15 | Field run | | 39 | 4.29 | 0.707 | 6.57 |

[1] The California Department of Agriculture Standard for well-colored tomatoes is 46 or lower; fairly well-colored, 47–67; and rejects, 68 or above.

type or other undetermined factors. Soluble solids content seemed to be independent of the ripeness levels used in this investigation.

**Pectic substances.** Changes in pectin content of early, middle, and late season tomatoes are indicated in Table 2.

The protopectin content of the fruit decreased slightly with ripening. As the fruit ripened, the protopectin content decreased from 30.9 to 23.4% of total pectin in early season, and from 26.8 to 23.7% in late season tomatoes.

The effect of ripeness level on the free galacturonic acid content of tomatoes is shown in Table 2. The galacturonic acid content increased slightly as the tomatoes ripened. Slightly more galacturonic acid was present in the riper fruit of the later season than in those of the early season.

Pectic substances were investigated in 5 varieties of California tomatoes which differ in shape and in resistance to Fusarium and Verticillium fungi. Samples were picked on the same day and precautions taken to select fruit of the same ripeness, thus minimizing factors which might alter their chemical composition. Results are shown in Table 3. Pear-shaped varieties were higher in total pectin and protopectin content than were the round-shaped varieties. Of the 3 round-shaped varieties studied, C58F1 and Pearson VF1 varieties were higher than the CPC No 2 variety in total and protopectin content. From the information presented in Table 3, it seems likely that resistance to fungi attack is not related to pectin content.

**Cellulose.** Although the relationship between pectic substances in tomatoes and consistency of the macerated product has been extensively investigated *(5, 8, 9, 10, 11, 14, 15, 21)*, relatively little attention has been given the role of cellulose. Experiments were carried out to study the effect of ripeness levels on the cellulose content of the canned juice. Results shown in Table 4 indicate that cellulose content varies only

**TABLE 4**

**Influence of ripeness level on cellulose content of canned tomato juice (improved Pearson variety, 1958 crop)**

| Ripeness level | Cellulose, % | | | | | |
|---|---|---|---|---|---|---|
| | Early season 9/8/58 | | Middle season 9/22/58 | | Late season 10/20/58 | |
| | Fresh basis | Dry basis | Fresh basis | Dry basis | Fresh basis | Dry basis |
| Pink | 0.280 | 3.94 | 0.275 | 3.59 | 0.250 | 3.68 |
| Firm | 0.265 | 3.71 | 0.280 | 3.66 | 0.245 | 3.65 |
| Canning-ripe | 0.280 | 3.94 | 0.265 | 3.48 | 0.245 | 3.48 |
| Soft-ripe | 0.270 | 3.91 | 0.275 | 3.62 | 0.255 | 3.55 |
| Field run | 0.265 | 3.72 | 0.275 | 3.63 | 0.278 | 3.66 |

**TABLE 2**

**Changes in pectin content of improved Pearson tomatoes during ripening (1958 crop)**

| Sample | Ripeness level | Total pectin | | Protopectin | | | Water soluble pectin | | | Galacturonic acid | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fresh basis, % | Dry basis, % | Fresh basis, % | Dry basis, % | % of total pectin, dry basis | Fresh basis, % | Dry basis, % | % of total pectin, dry basis | Fresh basis, % | Dry basis, % |
| | Early Season | | | | | | | | | | |
| 1 | Pink | 0.243 | 3.43 | 0.076 | 1.06 | 30.9 | 0.167 | 2.37 | 69.1 | 0.0108 | 0.152 |
| 2 | Firm | 0.252 | 3.43 | 0.074 | 1.00 | 29.2 | 0.178 | 2.43 | 70.9 | 0.0128 | 0.174 |
| 3 | Canning-ripe | 0.270 | 3.81 | 0.067 | 0.94 | 24.7 | 0.203 | 2.86 | 75.1 | 0.0153 | 0.215 |
| 4 | Soft-ripe | 0.218 | 3.16 | 0.051 | 0.74 | 23.4 | 0.166 | 2.42 | 76.6 | 0.0175 | 0.254 |
| 5 | Field run | 0.236 | 3.31 | 0.067 | 0.94 | 28.4 | 0.169 | 2.37 | 71.6 | 0.0152 | 0.213 |
| | Middle Season | | | | | | | | | | |
| 6 | Pink | 0.296 | 3.87 | 0.088 | 1.15 | 29.7 | 0.207 | 2.72 | 70.3 | 0.0076 | 0.100 |
| 7 | Firm | 0.270 | 3.53 | 0.069 | 0.90 | 25.5 | 0.201 | 2.64 | 74.8 | 0.0175 | 0.228 |
| 8 | Canning-ripe | 0.260 | 3.42 | 0.058 | 0.76 | 22.2 | 0.202 | 2.67 | 78.1 | 0.0193 | 0.253 |
| 9 | Soft-ripe | 0.244 | 3.22 | 0.063 | 0.84 | 26.1 | 0.180 | 2.39 | 74.2 | 0.0240 | 0.325 |
| 10 | Field run | 0.238 | 3.14 | 0.064 | 0.84 | 26.8 | 0.174 | 2.31 | 73.6 | 0.0330 | 0.441 |
| | Late Season | | | | | | | | | | |
| 11 | Pink | 0.263 | 3.88 | 0.070 | 1.04 | 26.8 | 0.192 | 2.84 | 73.2 | 0.0104 | 0.153 |
| 12 | Firm | 0.262 | 3.91 | 0.066 | 0.98 | 25.1 | 0.196 | 2.93 | 74.9 | 0.0148 | 0.220 |
| 13 | Canning-ripe | 0.258 | 3.67 | 0.061 | 0.87 | 23.7 | 0.196 | 2.80 | 76.3 | 0.0233 | 0.331 |
| 14 | Soft-ripe | 0.252 | 3.51 | 0.060 | 0.83 | 23.7 | 0.192 | 2.68 | 76.4 | 0.0268 | 0.373 |
| 15 | Field run | 0.265 | 3.89 | 0.070 | 1.02 | 26.2 | 0.195 | 2.87 | 73.8 | 0.0163 | 0.239 |

**TABLE 3**

**Pectin content of different tomato varieties (1958 crop)**

| Variety | Shape | Resistance to fungus | Total pectin | | Protopectin | | | Water soluble pectin | | | Galacturonic acid | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Fresh basis, % | Dry basis, % | Fresh basis, % | Dry basis, % | % of total pectin, dry basis | Fresh basis, % | Dry basis, % | % of total pectin, dry basis | Fresh basis, % | Dry basis, % |
| X | Pear | Verticillium | 0.296 | 4.72 | 0.099 | 1.58 | 33.5 | 0.196 | 3.13 | 66.3 | 0.0206 | 0.329 |
| San Marzano | Pear | None | 0.312 | 5.03 | 0.113 | 1.82 | 36.2 | 0.199 | 3.21 | 63.8 | 0.0238 | 0.384 |
| C 58 F1 | Round | Fusarium | 0.281 | 4.43 | 0.084 | 1.32 | 29.8 | 0.197 | 3.10 | 70.0 | 0.0217 | 0.342 |
| Pearson VF 1 | Round | Fusarium and Verticillium | 0.281 | 4.40 | 0.093 | 1.46 | 33.2 | 0.188 | 2.94 | 66.8 | 0.0203 | 0.318 |
| CPC No 2 | Round | Fusarium and Verticillium | 0.237 | 3.64 | 0.069 | 1.05 | 28.9 | 0.168 | 2.58 | 70.9 | 0.0198 | 0.304 |

slightly in juices canned from fruits of different ripeness levels. On a fresh basis, early and middle season juices were slightly higher in cellulose content than those from late season fruit. On a dry basis, however, very little difference was observed.

**Consistency.** Effect of ripeness level on consistency of canned tomato juice is shown in Table 5. The Brookfield reading decreased as spindle speed increased. A linear relationship was observed when apparent viscosity was plotted against spindle speed on a log-log scale. Juices made from firm-ripe tomatoes were thicker in consistency than those made from canning-ripe or soft-ripe tomatoes. In the literature, the Brookfield viscometer was used for determining consistency of tomato juice with spindle No 1 rotating at 20 rpm *(6, 30)*. Some

TABLE 5

**Effect of ripeness level on consistency of canned tomato juice (improved Pearson variety, 1958 crop)**

| Sample | Ripeness level | Apparent viscosity at 20° C | | | |
|---|---|---|---|---|---|
| | | Brookfield viscometer reading, centipoises[1] | | Pipette method[3] flow time in seconds | |
| | | Whole juice | Serum | Whole juice | Serum |
| | Early season | | | | |
| 1 | Pink | 448 | 8.7 | 43.5 | 25.4 |
| 2 | Firm | 426 | 8.0 | 37.4 | 24.3 |
| 3 | Canning-ripe | 377 | 5.6 | 33.1 | 23.4 |
| 4 | Soft-ripe | 322 | 4.2 | 31.0 | 23.0 |
| 5 | Field run | 388 | 6.1 | 32.5 | 23.8 |
| | Middle season | | | | |
| 6 | Pink | 550[2] | 15.0 | 49.2 | 27.3 |
| 7 | Firm | 510[2] | 12.3 | 45.1 | 25.3 |
| 8 | Canning-ripe | 382 | 11.0 | 34.3 | 24.0 |
| 9 | Soft-ripe | 270 | 8.0 | 33.0 | 23.7 |
| 10 | Field run | 477[2] | 11.0 | 34.5 | 23.9 |
| | Late season | | | | |
| 11 | Pink | 505[2] | 15.0 | 49.2 | 26.9 |
| 12 | Firm | 500[2] | 13.3 | 45.5 | 25.4 |
| 13 | Canning-ripe | 487 | 12.0 | 37.3 | 24.1 |
| 14 | Soft-ripe | 430 | 10.0 | 35.7 | 23.8 |
| 15 | Field run | 490[2] | 11.6 | 37.7 | 24.1 |

[1] A No 1 spindle was used at 20 rpm.
[2] Apparent centipoises derived by plotting on a log-log scale of data obtained with spindle No 2 at 2, 4, and 10 rpm.
[3] The flow time for distilled water = 20.5 seconds.

commercial juices had Brookfield readings ranging from 60 to 235 cps *(6, 30)*. Results obtained here on juices made from canning- and soft-ripe tomatoes agreed reasonably well with those in the literature. The pipette method revealed trends similar to those obtained by the Brookfield viscometer.

Both the Brookfield viscometer and the pipette registered lower consistency in tomato juice serum than in whole juice (Table 5), indicating that pulp is an important contributor to consistency. This agrees with the results reported by Kertesz and Loconti *(8)*.

**Importance of pectin to consistency.** To determine the relationship between consistency and pectin, the serum was separated from the tomato pulp by centrifugation. The pulp was washed with distilled water, extracted with 0.5% versene for 18 hr, washed again with distilled water, centrifuged to remove the supernatant, and then mixed with the serum to make up the original volume of the juice. Table 6 reveals that the consistency of all the juices so treated became thinner, indicating that the versene-soluble fraction of the pulp is an important consistency factor. Viscosity retention in the depectinized juice was 88.8% in the sample made from soft-ripe tomatoes, whereas that from firm-ripe fruit was only 62.7%. High protopectin content in the firm-ripe fruit may have caused a greater decrease in consistency after versene extraction.

## DISCUSSION

The importance of preheating temperature on pectic enzyme inactivation and consistency of tomato products has been demonstrated *(8, 11, 16, 17, 18, 25)*. Processing conditions and particle size were equally important factors *(6, 12, 23, 24)*.

TABLE 6

**Relative importance of cellulose and pectin to consistency of canned tomato juice (middle season crop, 1958)**

| Sample | Ripeness level | Cellulose in the juice, % | Total pectin in tomatoes, % | Brookfield viscometer reading, centipoises[1] | | Viscosity retention in depectinized juice, % |
|---|---|---|---|---|---|---|
| | | | | Tomato juice | Depectinized tomato juice | |
| 6 | Pink | 0.275 | 0.296 | 550[2] | 345 | 62.7 |
| 7 | Firm | 0.280 | 0.270 | 510[2] | 320 | 62.7 |
| 8 | Canning-ripe | 0.265 | 0.260 | 382 | 267 | 69.8 |
| 9 | Soft-ripe | 0.275 | 0.244 | 270 | 240 | 88.8 |

[1] A No 1 spindle was used at 20 rpm.
[2] Apparent centipoises derived by plotting on a log-log scale of data obtained with spindle No 2 at 2, 4, and 10 rpm.

The present investigation has demonstrated the relationship between ripeness level of fresh tomatoes and consistency of the canned juice. Both the Brookfield viscometer and the pipette indicated that juices made from soft-ripe tomatoes were thinner in consistency than those made from firm-ripe fruit. This might be partially explained by the higher protopectin content in firm-ripe tomatoes.

Galacturonic acid in tomato juice constitutes only a small portion of the total uronide material in the juice. Since the galacturonic acid content was slightly higher in juices made from ripe fruit, it is possible that pectic enzymes in tomatoes contribute to galacturonic acid formation in tomato juice. However, the presence of mold polygalacturonase in soft-ripe tomatoes could also be a factor.

Kertesz and Loconti *(8)* stated that viscous serum is indispensable for tomato juice of desirable consistency. They also indicated that the amount and character of the suspended solids as well as their pectic constituents are important to consistency. Results obtained here confirm their findings.

Whittenberger and Nutting *(29)* reported that the flesh cell is the principal structural element in tomato juice, and that the cell wall of the flesh cell is the structure most closely related to consistency. In a recent publication, Whittenberger and Nutting *(30)* studied the high viscosity of cell wall suspensions prepared from tomato juice and stated that electrolyte content and tomato cell walls are related to juice viscosity. Of the substances which comprise tomato cell walls, cellulose was reported to be most closely related to juice viscosity. However, results obtained here by chemical analyses of the cellulose content in tomato juice seem to indicate that such a relationship does not hold. The role of cellulose may be explained, however, by the hypothesis that since cell wall characteristics are determined by the way cellulose is combined with pectin or other high molecular-weight polymers which influence the physical condition of the insoluble particles, the consistency of the canned juice will be influenced.

The present investigation indicates that fruit ripeness and varietal characteristics are important to juice consistency. Since a gross consistency that is too thick or too thin is undesirable, proper control of fruit ripeness and processing conditions are necessary to obtain juices of optimum consistency.

## SUMMARY

Tomato juices were canned during the early, middle, and late seasons at four different stages of ripeness as judged by skin color and the Agtron E colorimeter. The effect of ripeness level on pectin, cellulose, and consistency of canned juices was investigated.

Total pectin decreased with ripening. Protopectin decreased in proportion to an increase in water-soluble pectin, indicating solubilization of protopectin as the fruit matured. Pear-shaped tomato varieties contained higher pectin than round-shaped varieties. The varietal characteristics of resistance to Fusarium and Verticillium wilt appeared to be independent of pectin content.

The Brookfield viscometer and the pipette were used to determine consistency. Juices made from firm-ripe tomatoes were thicker in consistency than those made from canning-ripe or soft-ripe tomatoes. Consistency was lowered when the pulp was depectinized with versene and then recombined with the serum. This indicates that pectic substance in the tomato pulp is an important contributor to juice consistency. Various factors influencing tomato juice consistency are discussed.


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# Factors Contributing to the Breakdown of Frozen Sliced Strawberries[a]

(Manuscript received May 17, 1960)

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AN INCREASING CONCERN in recent years by consumers and the strawberry industry about the breakdown in character or texture of frozen sliced strawberries led to an investigation of the basic causes of this condition.

Many growing and handling variations have been shown to be related to firmness of strawberries, both in the fresh and in the frozen product. Either softer varieties of strawberries or over-ripe fruit was associated with poor character in frozen strawberries *(8, 12)*. A direct correlation was shown between dry weight and firmness in 10 varieties of strawberries that were separated into four firmness groups *(6)*. Rainfall, humidity, and time of harvest influenced the firmness of fresh and frozen fruit, and fruit was softer immediately after a rain and late in the season *(2, 10)*. The application of fertilizers lowered the soluble and total solids in fresh strawberries and increased the size. Larger fruit were softer than smaller fruit which indicated that an increase in size of fruit from high nitrogen fertilizer was a factor related to firmness *(3)*.

Mold infections on strawberries were higher when harvested from heavy foliaged plants and during periods of high humidity. Injuries caused by careless picking and handling increased mold counts, especially after post-harvest storage *(2, 10)*. Length of time between pickings had less effect on mold counts than did rainfall *(1)*.

The removal of field heat from fresh strawberries was shown to greatly retard breakdown. Strawberries were quickly destroyed by a high rate of metabolic activity at 86° and 100° F. Firmness of strawberries was increased by decreasing the temperature from 72° to 36° F when measured by a plunger-type tester, but a crushing-type tester revealed no major changes in firmness, indicating that the epidermis of the fruit was the area toughened by cooling *(11)*.

The addition of pectin, sodium alginate and other colloids to strawberries before freezing has been used to increase the firmness with variable results. Colloids were less effective in firming slices in dry sugar packs than in syrup packs *(7, 14)*. These variations were probably due to variety, maturity and method of application.

Types of fruit-sugar mixers were shown to influence character or texture of the final frozen sliced strawberries. The ribbon-type mixer produced 13% mushy slices and the screw-type mixer 20% as compared to a two-stage filling operation with syrup which produced only 3% mushy slices in the final product *(13)*.

The rate of freezing of the packaged product greatly affected color oxidation and character, or mushiness *(5, 8)*. Furthermore, the changes in temperature during storage of the frozen product influenced texture, appearance and palatability.

The objectives in this experimental work were to study the effects of variety, date of harvest, mold inhibitor sprays, holding time and temperature after harvest, rate of freezing, method of slicing, and length of time of mixing the fruit and sugar on character or mushiness of the frozen-thawed product.

## MATERIALS AND METHODS

Four varieties of strawberries—Northwest, Marshall, Siletz, and Puget Beauty—were obtained for the various experiments. The fruit from 3 harvests of a replicated field experiment, which consisted of the 4 varieties, was one phase of the study. A second phase consisted of the fruit of Northwest and Marshall varieties from 2 harvests in which field applications of the mold inhibitor N-trichloromethylthiotetrahydrophthalimide (Captan) were used. Random samples of the individual lots were held as long as 18 hr at pilot plant temperatures (60°–80° F) prior to preparation for freezing. A third phase was accomplished by using freshly harvested, uniform lots of fruit of Siletz variety to study holding time and temperature, method of slicing, rate of freezing and length of time of mixing the fruit with sugar prior to freezing.

**Preparation for freezing.** The fruit was prepared for freezing by washing in a McLauchlan berry washer and draining on a screen to remove water. All of the fruit was sliced with a high-speed, serrated blade slicer (McLauchlan) except where slicing berries was the variable. Another method of slicing consisted of slicing berries into halves by hand to minimize bruising of slices. A third method was by the use of a slow-speed centrifugal slicer. The mechanical slicers were set to slice 7/16 inch in thickness.

Sugar was added at the rate of 1 lb to 4 lb of fruit. The fruit and sugar were placed directly into a large-mouthed, 5-gal glass jar which had been fitted with crossbars to mix the fruit during rotation. After closing the jar, it was rotated horizontally on rollers that were driven by a motor set to rotate at a speed of 26 rpm. A mixing time of 1 min was used as the standard for all lots except those where mixing time was increased. The mixed fruit and sugar was filled into 1 lb fiberboard, metal-end cartons; then, closed by a semi-automatic metal-end capper and frozen to 0° F in 3 to 4 hr in a freezing room at −18° F.

The 3 rates of freezing for the phase of the study on freezing rate were 1, 4 and 18 hr for the center of the packaged product to reach 0° F.

**Analytical.** The resistance to puncture was measured on the fresh fruit by a pressure tester with a 3/16 inch diameter plunger. One lb samples of fresh fruit were ground in a Waring blender for the determination of soluble and total solids, pH, total acidity, and pectin fractions. Soluble solids were deter-

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[b] Presented at the Twentieth Annual Meeting of the Institute of Food Technologists, May 17, 1960.

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mined with a refractometer. Total solids were obtained on 10 g samples of blended strawberries after drying for 24 hr at 158° F under 27 in of vacuum. Total acidity was determined by titrating 10 g samples with 0.2 N NaOH to an end-point of pH 8.0 with the aid of a pH meter and stirring device. Pectin fractions were analyzed by the method of Dietz and Rouse *(4)*.

The frozen product was analyzed after 6 months of storage at 0° F. Samples of fruit were thawed for 2 hr in a water-bath at 68° F prior to determination of drained weight, mushy slices, viscosity of syrup, resistance to shear, soluble and total solids, total acidity, and pectin fractions.

Drained weight was recorded as the weight of strawberries remaining on a No. 8 mesh screen after 3 min of draining. Mushy or disintegrated slices were separated from the intact slices with a fork during the draining period. These were weighed after the removal of intact slices. A sample of the syrup was filtered through 2 thicknesses of cheesecloth before the determination of the rate of flow from a size 300 Oswald pipette at a constant temperature of 68° F.

Resistance to shear was then determined on a 150 g sample of the thawed drained fruit by the use of a Kramer shear-press which had been modified to include a 50-lb pressure ring, exciter demodulator, differential transformer indicator, and a two-axis recorder.

The fruit and syrup were recombined and ground in a Waring blender for the determination of soluble and total solids, total acidity, and pectin fractions as previously described.

Conventional methods were used for the analysis of variance on these data.

## RESULTS AND DISCUSSION

Variety of strawberries, date of harvest, and length of time of mixing the fruit and sugar were found to be the most important factors involved in the breakdown of frozen, sliced strawberries. Rate of freezing of the packaged product, mold inhibitor sprays, and the time and temperature of holding the fresh fruit prior to preparation for freezing were of lesser importance.

Although analyses were made on the fresh fruit in the separate phases of the study, these data will not be discussed except in instances where an explanation of data on the frozen product warrants involving the raw material.

**Varieties and harvests.** The graphs for the 4 varieties indicate that there were marked differences between varieties in firmness, and the mushiness of the thawed frozen strawberries (Figures 1–6). Marshall frozen strawberries had more mushy slices in 2 of the 3 harvests, lower viscosity of syrup, and lower water-soluble pectin content than the other varieties. Siletz was markedly higher in total acidity in the 3 harvests. Fresh fruit firmness of Puget Beauty was greater than Marshall by the puncture test, yet in the frozen product the shear-press measurements were higher in the Marshall variety.

There was an increase in per cent mushy slices in the second and third harvests in most instances as compared to the first harvest (Figure 1). Viscosity of syrup and shear-press work declined in the second and third harvests in all instances except two (Figures 2, 5, 6). The maturity of the fruit, or condition at harvest probably accounted for most of the harvest differences.

**Mold inhibitor spray and holding prior to freezing.** The control lots of the Marshall variety were signifi-

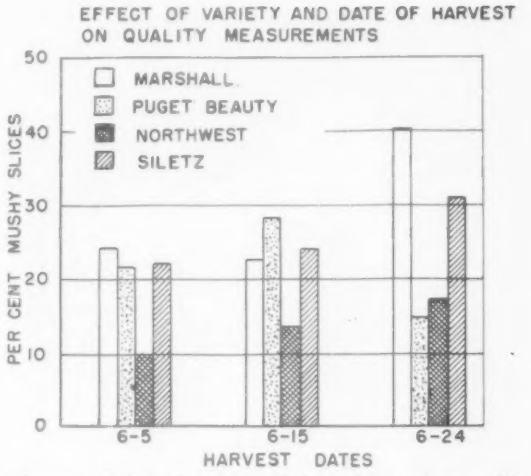


Figure 1. Effect of variety and date of harvest on quality measurements.

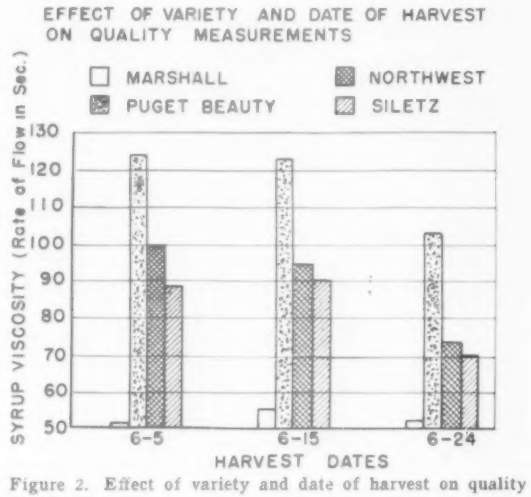


Figure 2. Effect of variety and date of harvest on quality measurements.

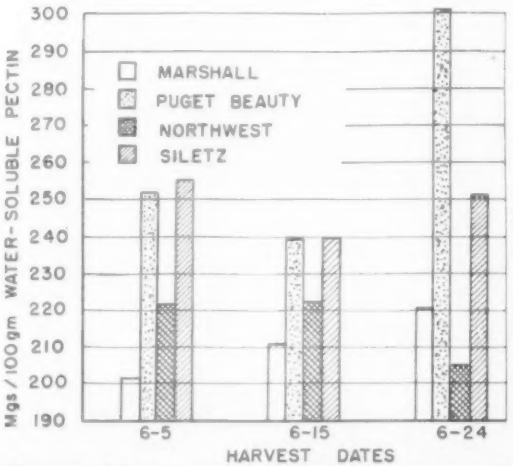


Figure 3. Effect of variety and date of harvest on quality measurements.

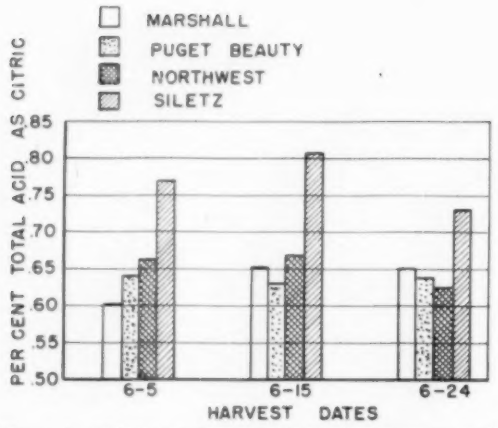


**Figure 4. Effect of variety and date of harvest on quality measurements.**

cantly lower in viscosity when processed immediately after harvest (Table 1). No other significant differences were obtained between the lots of fruit that were sprayed with Captan, although the shear-press work and the total acidity were lower on both control and Captan lots that were held for 18 hr at room temperature before freezing. Also, water-soluble pectin content was higher on these lots.

Per cent mushy slices and water-soluble pectin content were higher in fruit from the second harvest. Furthermore, viscosity of syrup, total acidity and shear-press measurements were lower on fruit from the second harvest (Table 1). Again, the important differences resulted from the time of harvest. Holding the fruit prior to freezing was not too detrimental to firmness of slices when the fresh fruit was in good condition at harvest.

The fruit of Northwest variety was lower in total acidity, viscosity of syrup and shear-press measurements when held 18 hr at room temperature (Table 2). Per cent mushy slices increased more in the control as a result of holding than in the lots sprayed with

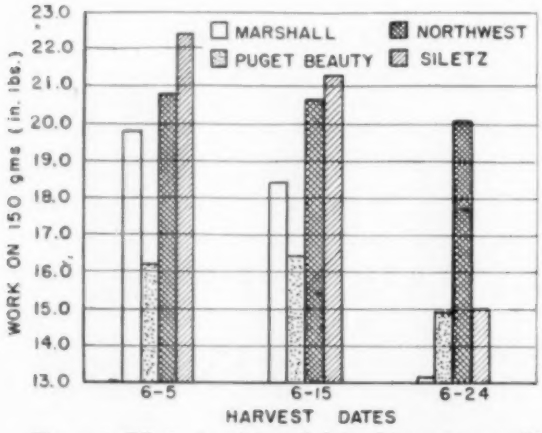


**Figure 5. Effect of variety and date of harvest on quality measurements.**

Captan. The fresh fruit from the sprayed lots was better in appearance than the controls, although definable differences were not obtained in either the fresh or the frozen product analyses, except in a few instances.

Harvest differences were again the most pronounced. Per cent mushy slices and shear-press measurements were lower on fruit of the second harvest (Table 2). Viscosity of syrup, water-soluble pectin content and total acidity were significantly higher in fruit of the second harvest of the Northwest variety.

**Type of slicing, length of time of mixing and rate of freezing.** The fruit that was sliced by hand was significantly lower in per cent mushy slices than fruit that

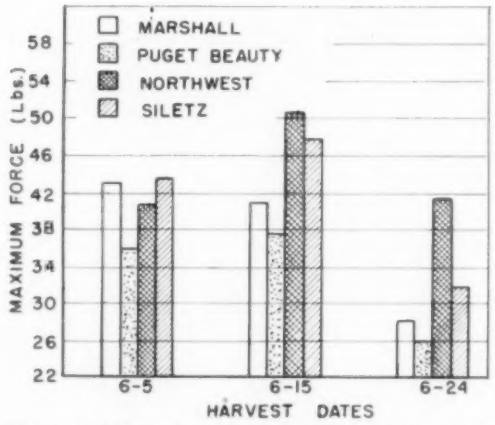


**Figure 6. Effect of variety and date of harvest on quality measurements.**

was sliced mechanically (Table 3). Fruit that was sliced on the high-speed, serrated blade slicer was more mushy than fruit sliced on the slow-speed centrifugal slicer, although differences were not significant.

Significant increases in viscosity of syrup and per cent mushy slices were apparent when fruit and sugar were mixed for 15 and 30 min. Since most of the fruit

**TABLE 1**

**Effect of mold inhibitor spray, harvest date and holding prior to freezing on quality measurements of Marshall strawberries**

**Means of treatment and harvest effects**

| Treatments | % Mushy slices | Rate of syrup flow | Mgs/100 g water-sol pectin | % Total acid as citric | Shear-press | |
|---|---|---|---|---|---|---|
| | | | | | Work on 150 g | Max force |
| | | (sec) | | | (in lb) | (lb) |
| Control | 31.2 | 56.5 | 269 | .60 | 14.9 | 33.3 |
| Captan | 28.5 | 69.2 | 269 | .61 | 13.7 | 30.9 |
| Control (H)* | 27.7 | 62.3 | 313 | .60 | 12.3 | 30.3 |
| Captan (H) | 27.4 | 62.3 | 305 | .60 | 12.9 | 30.4 |
| LSD.05 | NS | 7.7 | NS | NS | NS | NS |
| LSD.01 | NS | NS | NS | NS | NS | NS |
| Harvest date | | | | | | |
| 6/3 | 26.5 | 73.5 | 230 | .61 | 15.8 | 35.0 |
| 6/18 | 30.9 | 51.6 | 347 | .59 | 11.1 | 27.4 |
| LSD.05 | NS | 5.4 | 49 | NS | 3.8 | 0.5 |
| LSD.01 | NS | 7.7 | 90 | NS | 7.1 | 1.0 |

* Held.

TABLE 2

**Effect of mold inhibitor spray, harvest date and holding prior to freezing on quality measurements of Northwest strawberries**

**Means of treatment and harvest effects**

| Treatments | % Mushy slices | Rate of syrup flow | Mgs/100 g water-sol pectin | % Total acid as citric | Shear-press | |
|---|---|---|---|---|---|---|
| | | | | | Work on 150 g | Max force |
| | | *(sec)* | | | *(in lb)* | *(lb)* |
| Control | 19.5 | 92.5 | 193 | .61 | 30.7 | 61.1 |
| Captan | 18.6 | 110.5 | 197 | .60 | 29.8 | 56.0 |
| Control (H)* | 24.9 | 80.8 | 199 | .56 | 25.6 | 55.6 |
| Captan (H) | 19.7 | 84.8 | 194 | .56 | 24.7 | 50.6 |
| LSD .05 | NS | 14.8 | NS | .03 | NS | NS |
| LSD .01 | NS | NS | NS | .05 | NS | NS |
| Harvest date | | | | | | |
| 6/12 | 22.3 | 80.4 | 180 | .54 | 29.7 | 57.5 |
| 6/18 | 19.0 | 103.9 | 211 | .63 | 25.7 | 54.1 |
| LSD .05 | NS | 10.5 | 23 | .02 | NS | NS |
| LSD .01 | NS | 19.3 | NS | .03 | NS | NS |

* Held.

slices were partially or wholly disintegrated there was a significant decrease in shear-press measurements of firmness as compared to the control lot.

Sliced fruit that was packed in syrup was less mushy than the control lot, but water-soluble pectin content, total acidity, and viscosity of syrup were lower due to added water in the syrup.

A slower rate of freezing increased the firmness of slices as measured by the shear-press, although the viscosity of the syrup decreased. Since more water was withdrawn from the fruit when frozen slowly this caused a toughening of the tissues; thus firmer slices, more drained weight loss, and lower viscosity of syrup. There were no differences in water-soluble pectin content and total acidity as a result of freezing rate. The control lot (4 hr) was significantly higher in per cent mushy slices than either the slow or the fast rate of freezing (Table 3).

**Holding time and temperature prior to freezing.** The per cent mushy slices increased with an increase in holding time; whereas, there was a decrease in viscosity of syrup (Table 4). Other differences were not clearly definable although shear-press work indicated an increase in firmness when holding time was increased. This increase was probably due to a toughening of the epidermis due to dehydration during the holding period.

TABLE 3

**Effect of slicing, mixing and rate of freezing on quality measurements**

**Means of treatments**

| Treatments | % Mushy slices | Rate of syrup flow | Mgs/100 g water-sol pectin | % Total acid as citric | Shear-press | |
|---|---|---|---|---|---|---|
| | | | | | Work on 150 g | Max force |
| | | *(sec)* | | | *(in lb)* | *(lb)* |
| Control, high speed slicer | 26.1 | 64.0 | 205 | .67 | 22.1 | 32.7 |
| Slow speed slicer | 18.6 | 68.0 | 206 | .63 | 18.6 | 27.2 |
| Sliced by hand | 8.5 | 69.0 | 207 | .66 | 19.0 | 25.4 |
| Coarse sugar | 28.1 | 67.5 | 208 | .69 | 15.8 | 21.9 |
| 15 min mix | 30.9 | 82.5 | 196 | .67 | 15.0 | 22.4 |
| 30 min mix | 45.4 | 89.5 | 218 | .68 | 12.6 | 24.6 |
| 50% syrup | 15.5 | 33.5 | 164 | .55 | 16.7 | 22.8 |
| Frozen to 0° F in 4 hr | 21.4 | 61.0 | 172 | .68 | 16.7 | 29.5 |
| Frozen to 0° F in 1¼ hr | 15.0 | 65.0 | 178 | .67 | 17.5 | 27.2 |
| Frozen to 0° F in 18 hr | 15.9 | 52.0 | 181 | .68 | 22.6 | 36.0 |
| LSD .05 | 4.8 | 6.8 | 15 | .02 | 3.1 | 4.5 |
| LSD .01 | 6.9 | 9.8 | 21 | .03 | 4.4 | 6.4 |

TABLE 4

**Effect of temperature and time of holding prior to freezing on quality measurements**

**Means of treatments**

| Holding treatments | % Mushy slices | Rate of syrup flow | Mgs/100 g water-sol pectin | % Total acid as citric | Shear-press | |
|---|---|---|---|---|---|---|
| | | | | | Work on 150 g | Max force |
| | | *(sec)* | | | *(in lb)* | *(lb)* |
| Control | 18.3 | 75.0 | 158 | .63 | 22.5 | 52.0 |
| 8 hr 34° F | 27.9 | 62.5 | 159 | .64 | 20.6 | 53.7 |
| 8 hr 78° F | 22.5 | 54.5 | 170 | .65 | 21.3 | 53.8 |
| 8 hr field & pilot plant (60° to 78° F) | 28.1 | 67.5 | 166 | .67 | 18.5 | 49.4 |
| 18 hr 34 °F | 31.5 | 66.5 | 164 | .62 | 23.3 | 51.7 |
| 18 hr pilot plant | 36.6 | 47.5 | 170 | .67 | 25.7 | 52.2 |
| 8 hr field, 18 hr pilot plant | 31.2 | 47.5 | 165 | .61 | 30.3 | 57.5 |
| LSD .05 | 7.6 | 4.8 | NS | NS | 6.0 | NS |
| LSD .01 | 11.5 | 7.1 | NS | NS | NS | NS |

## SUMMARY

A study was made to assess the importance of variety, date of harvest, mold inhibitor spray, holding time and temperature after harvest, rate of freezing, method of slicing, and length of time of mixing fruit and sugar in relation to the breakdown in character of frozen sliced strawberries.

The four varieties—Northwest, Marshall, Siletz and Puget Beauty—were found to react differently to freezing although fresh fruit quality was maximal. Percentages of mushy slices in the frozen product were lower in the Northwest variety. Also, shear-press measurements of firmness were higher than in the other varieties.

Wherever harvest was involved, there was an increase in the per cent of mushy slices and water-soluble pectin content in the frozen product that was prepared from fruit harvested beyond mid-season. Furthermore, there was a decrease in the viscosity of syrup and shear-press measurements of firmness of the thawed frozen product. Maturity probably had a great deal to do with the effect of date of harvest.

An increase in mixing time beyond the time necessary to dissolve the sugar significantly increased the per cent of mushy slices and syrup viscosity of the thawed frozen slices of Siletz variety. Shear-press measurement of firmness was decreased.

In the Siletz variety, there was a steady increase in the per cent of mushy slices when fresh fruit was held either at 34° F, or at room temperature prior to processing. Syrup viscosity decreased during holding up to 24 hours.

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# Inhibition of Polygalacturonase in Brined Cherries[a, b]

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(Manuscript received May 15, 1960)

Brined cherries are the raw products from which maraschino type cherries are prepared. The brine, in general, is made up of 1¼% sulfur dioxide, a preservative and bleaching agent, and ¾% calcium hydroxide or calcium chloride. The calcium acts as a firming agent *(11)*.

In contrast to ordinary processed foods the main consideration in brined cherries is the texture or firmness of the fruit, the color and flavor being added artificially during the maraschino manufacturing process *(1)*. The firmness of the cherries depends primarily on the natural pectin present *(5)*, and when this pectin is destroyed the cherries become soft *(6)*. This softening resulted in a loss of ¾ million dollars in one season for one locality alone *(11)*. A typical result of pectin degrading enzyme action on brined cherries is shown in Figure 1.

The destruction of pectin has been found to be due to the action of the pectin degrading polygalacturonase *(7)*. The source of this enzyme has not yet been determined, although microorganisms and/or plant diseases have been suspected *(9)*.

While the search for the source of polygalacturonase continues, its possible inhibitors have been investigated. The enzyme apparently is not inhibited by sulfur dioxide, calcium hydroxide, or calcium chloride. Since calcium chloride does not neutralize the sulfurous acid, the resulting brine has a lower pH. Most enzymes are known to be inactive at extremely low and high pH's *(8)*.

Experiments have shown that Nacconol[d] (a commercial preparation of sodium alkyl aryl sulfonate) is an effective inhibitor of polygalacturonase in model systems *(10)*. This paper presents evidence for the effectiveness of other commercial preparations of alkyl aryl sulfonate as inhibitors of polygalacturonase, and as inhibitors of naturally occurring pectic enzymes in brined cherries. It also describes the de-

[a] Presented before the Twentieth Annual Meeting of the Institute of Food Technologists, San Francisco, California, May 16, 1960.

[b] Approved for publication by the Director of the Oregon Agricultural Experiment Station as Technical Paper No. 1324. Contribution of the Department of Food and Dairy Technology.

[c] Present address: Department of Agricultural Chemistry, Oregon State College, Corvallis, Oregon.

[d] Manufactured by National Aniline Division, Allied Chemical and Dye Corp., New York, N. Y.

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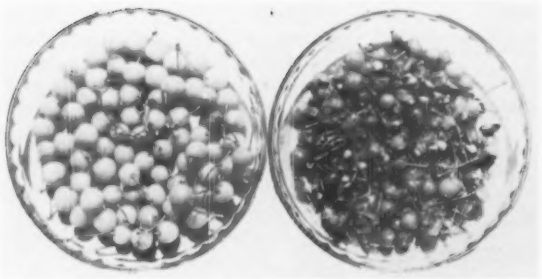

**Figure 1. Left: Normal brined cherries. Right: Cherries which were acted upon by pectin-degrading enzyme during brining.**

termination of residual alkyl aryl sulfonate in leached brined cherries using radioisotope tracer techniques and the effects of the residue on the finished maraschino cherries.

## EXPERIMENTAL PROCEDURE

### 1. Measurement of polygalacturonase activity

Polygalacturonase activity was measured by the Steele and Yang "drop-plate" method *(10)*, a modification of the "cup-plate" technique developed by Dingle, Reid and Solomons *(3)*.

A polygalacturonic acid substrate medium was prepared by slowly suspending 0.25% (w/v) of polygalacturonic acid (Exchange Lemon Products Co., No. 491) in distilled water with constant stirring. Next, 0.25% ammonium oxalate, 1% potassium biphthalate and 0.1% thymol preservative (all w/v) were added and dissolved. The pH was adjusted to 4.0 with 0.1 N sodium hydroxide. The medium was then transferred to an Erlenmeyer flask, 2% bacto agar (Difco) added, and sterilized by autoclaving for 15 min at 15 psig. Then 25 ml aliquots were transferred to 15 × 150 mm Petri dishes and allowed to solidify.

For each test, one drop (approximately 0.05 ml) of the test solution was placed on the flat surface of the test plate. The plate was then incubated for 20 hr at 37° C. At the end of the incubation period the plate was developed by flooding it with 2 N hydrochloric acid, allowing it to stand for 5 min and then rinsing with distilled water. Polygalacturonase activity was indicated by formation of a clear zone, and the extent of this activity was estimated by measuring the diameter of the zone formed. The average diameter of triplicate determinations was used for the establishment of data in all experiments.

### 2. Effect of pH on polygalacturonase activity

*A. In buffer solutions*

In studies on the activity of Pectinol [c] M enzyme solutions at different pH's, Clark and Lubs *(2)* buffer solutions were prepared covering the pH range from 1.0 to 8.0. A concentration of 0.1% of Pectinol M was used throughout the experiment.

The Clark and Lubs buffers used contained, HCl and KCl at pH values from 1.0 to 3.0, HCl and potassium acid phthalate at pH values from 3.0 to 5.0, and NaOH and potassium acid phthalate at the higher pH values. All buffer solutions were prepared using distilled water and their pH values verified with a Beckman Model G pH meter.

*B. In cherry brines*

The pH of cherry brines was varied by the use of different combinations of $Ca(OH)_2$ and $CaCl_2$ in 1¼% $SO_2$ solutions. Aqueous solutions without $SO_2$ were also prepared. By this method, pH's ranging from 1.2 to 12.5 were obtained.

Pectinol M in the concentration of 0.1% was added to each solution and inhibition observed after 24 hr.

[c] Pectinol is the trade name for the pectic enzyme preparations manufactured by Rohm and Haas Company, Philadelphia, Pennsylvania.

### 3. Inhibition of polygalacturonase by commercial preparations

To compare the effectiveness of other commercial alkyl aryl sulfonates with Nacconol, the following detergents were used:

Nacconol NR (40% active)
Nacconol NRSF (92.5%)
Oronite [f] (40% active)
HD-90 [g] (90% active)
Ultrawet [h] (40% active)

All the alkyl aryl sulfonates were tested at the concentration of 0.025% in a standard $SO_2$-$Ca(OH)_2$ brine with 0.1% Pectinol M added. For comparative purposes, a brine without any inhibitor was also tested. Cherries of the Bing variety were brined according to the prevailing commercial practice *(15)*. Their firmness was determined after 45 days with a Chatillon push-pull penetrometer [i] with a scale of 0-500 g. A 0.065-inch needle was used and 20 readings were obtained from each batch of brined cherries.

### 4. Inhibition of naturally-occurring pectic enzymes in brined cherries

Whereas enzyme activity in cherries was prevalent and strong in 1957, it was light and rare in the subsequent years. Cherries, fresh or brined, with positive enzyme test became hard to find. During the 1959 season, 5 brined cherry samples with a positive natural-enzyme test were obtained from a commercial brinery. These were the only cherries (fresh or brined) from the 1959 season which have been known to give a positive enzyme test in Washington, Oregon, and California. These cherries were all in soft and mushy condition. They had been in the brine for approximately four months.

The cherries and their brines were tested for enzyme activity. Nacconol NR in the concentration of 0.01% was then added to the brines and enzyme activity again evaluated after 24 hr.

### 5. Tracing of alkyl aryl sulfonate

The techniques used involved the radioactive labeling of Nacconol NR with tritium ($H_3$) by the electrophilic substitution method. The radioactive Nacconol was then added, in the concentration of 0.01%, to the cherry brine. Standard cherry brine of 1¼% $SO_2$ and ¾% $Ca(OH)_2$ was also used. The brined cherries, after 60 days, were leached in running water for 24 hr. They were then pitted and assayed for their radioactivity with a Tri-Carb liquid scientillation counter manufactured by the Packard Instrument Company of Chicago, Illinois. The amount of Nacconol remaining in the cherries was then calculated.

### 6. Effect of alkyl aryl sulfonate on finished maraschino cherries

In this experiment, Bing and Royal Anne cherries were observed after addition of 0.1% Nacconol NR to the brine at the time of brining. The cherries were left in contact with the detergent for 8 months, then processed into maraschino cherries by the method suggested by Filz and Henney *(4)*, and compared with control samples.

## RESULTS

### 1. Effect of pH on polygalacturonase activity

The effect of pH on enzyme activity in buffer solutions is shown in Table 1. The per cent activity is based on the highest activity obtained at pH 5.6 with no time lapse which is designated as 100% activity.

The effect of pH and brine formulation on enzyme activity is shown in Table 2. The per cent enzyme activity is based on the highest activity obtained at pH 2.0 with no time lapse which is designated as 100% activity.

[f] Manufactured by Oronite Chemical Co., San Francisco, California.
[g] Manufactured by Pilot Chemical Co., Whittier, California.
[h] Manufactured by Atlantic Refining Co., Philadelphia, Pa.
[i] Manufactured by John Chatillon & Sons, New York, N. Y.

TABLE 1

**Effect of pH on enzyme activity in buffer solutions**

| pH | % Enzyme activity | | |
|---|---|---|---|
| | After 2 hours | After 2 days | After 28 days |
| 1.0 | 0 | 0 | 0 |
| 1.6 | 75 | 0 | 0 |
| 2.0 | 84 | 66 | 0 |
| 2.6 | 84 | 70 | 45 |
| 3.0 | 89 | 86 | 64 |
| 3.6 | 91 | 86 | 82 |
| 4.0 | 90 | 91 | 86 |
| 4.6 | 93 | 91 | 86 |
| 5.0 | 91 | 91 | 84 |
| 5.6 | 95 | 91 | 84 |
| 6.0 | 95 | 93 | 84 |
| 8.0 | 98 | 82 | 54 |

### 2. Inhibition of polygalacturonase by commercial preparations

The effectiveness of 5 commercial alkyl aryl sulfonates as inhibitors of polygalacturonase is shown in Table 3 in terms of cherry firmness.

### 3. Inhibition of naturally-occurring pectic enzymes in brined cherries

Nacconol NR in the concentration of 0.01% completely inhibited the naturally-occurring pectic enzymes in 24 hr as shown in Table 4. Both the cherries and the brines were tested for enzyme activity before and after the Nacconol treatment. The enzyme activity is based on that of 0.1% Pectinol M which is designated as 100.

TABLE 2

**Effect of pH and brine formulation on enzyme activity**

| pH | Brine formulation | | | % Enzyme activity | | |
|---|---|---|---|---|---|---|
| | % $SO_2$ | % $Ca(OH)_2$ | % $CaCl_2$ | After 1 hr | After 6 hr | After 24 hr |
| 4.8 | 0 | 0 | 0.7 | 83 | 83 | 83 |
| 12.5 | 0 | 0.7 | 0 | 50 | 0 | 0 |
| 1.2 | 1.25 | 0 | 0 | 50 | 33 | 0 |
| 2.1 | 1.25 | 0.7 | 0 | 83 | 72 | 78 |
| 2.1 | 1.25 | 0.6 | 0.1 | 83 | 72 | 78 |
| 2.0 | 1.25 | 0.5 | 0.2 | 72 | 67 | 55 |
| 1.8 | 1.25 | 0.4 | 0.3 | 67 | 55 | 33 |
| 1.7 | 1.25 | 0.3 | 0.4 | 67 | 61 | 22 |
| 1.5 | 1.25 | 0.2 | 0.5 | 55 | 44 | 0 |
| 1.4 | 1.25 | 0.1 | 0.6 | 50 | 44 | 0 |
| 1.2 | 1.25 | 0 | 0.7 | 44 | 22 | 0 |

TABLE 3

**Chatillon penetrometer tests of brined cherries treated with commercial alkyl aryl sulfonate**

| No. treatments | Treatments | | | | |
|---|---|---|---|---|---|
| | Nacconol NR | Nacconol NRSF | Oronite | HR-90 | Ultrawet |
| 150 | 140 | 355 | 320 | 180 | 320 |
| 55 | 270 | 330 | 260 | 200 | 375 |
| 40 | 405 | 275 | 225 | 225 | 320 |
| 35 | 245 | 265 | 295 | 260 | 280 |
| 315 | 225 | 205 | 180 | 285 | 200 |
| 205 | 145 | 235 | 260 | 260 | 280 |
| 80 | 215 | 285 | 205 | 200 | 220 |
| 0 | 300 | 185 | 240 | 350 | 315 |
| 0 | 340 | 290 | 225 | 305 | 150 |
| 15 | 260 | 215 | 310 | 240 | 160 |
| 115 | 280 | 230 | 235 | 300 | 295 |
| 10 | 245 | 165 | 145 | 300 | 200 |
| 160 | 140 | 285 | 270 | 295 | 115 |
| 0 | 150 | 220 | 175 | 390 | 280 |
| 100 | 235 | 350 | 180 | 180 | 295 |
| 50 | 135 | 255 | 210 | 245 | 190 |
| 50 | 140 | 260 | 210 | 125 | 300 |
| 0 | 315 | 290 | 140 | 280 | 235 |
| 20 | 260 | 285 | 200 | 240 | 475 |
| 120 | 275 | 280 | 345 | 295 | 225 |
| Av. 76 | 236 | 263 | 232 | 260 | 261 |

TABLE 4

**Enzyme activity of brined cherries before and after nacconol treatment**

**Index: 100=0.1% pectinol M**

| Sample No | Cherries | | Brine | |
|---|---|---|---|---|
| | Before | After | Before | After |
| 1 | 67 | 0 | 72 | 0 |
| 2 | 69 | 0 | 78 | 0 |
| 3 | 131 | 0 | 139 | 0 |
| 4 | 125 | 0 | 128 | 0 |
| 5 | 97 | 0 | 119 | 0 |

### 4. Tracing of alkyl aryl sulfonate

Radioactive isotope tracer techniques revealed that 3% of the Nacconol added to the cherry brine remained in the cherries after they were leached in running water for 24 hr.

Based on this experiment, it is seen that the actual quantity of alkyl aryl sulfonate remaining in the brined and washed cherries is quite small. When 0.01% of Nacconol NR is added to the brine, assuming that in the brining process the cherries and brine are present in equal quantity, the Nacconol concentration after equilibrium becomes 0.005%. If 3% of the added Nacconol remained in the cherries, as indicated in the radioactive isotope assay experiment, only $0.03 \times 0.005$ or 0.00015% actually remained in the fruit. Since Nacconol NR contains only 40% active alkyl aryl sulfonate, the residual alkyl aryl sulfonate in the cherries is $0.00015 \times 0.40 = 0.00006\%$ or 0.6 ppm.

### 5. Effect of alkyl aryl sulfonate on finished maraschino cherries

The Nacconol-treated cherries, after processing into maraschino cherries were examined by the authors in comparison with the untreated cherries. No difference was observed in texture, flavor, and color of the finished maraschino product.

## DISCUSSION

The pH of the brine has a great effect on the activity of polygalacturonase. The enzyme was inactivated rapidly in buffer solutions at pH 1.0 and 1.6, and less rapidly at pH 2.0. At pH 2.6 and above, the inactivation, if any, was very slow. There was a small loss of activity for this enzyme in all cases after 28 days.

The pH of cherry brines has the identical effect on polygalacturonase as that of the buffer solutions. At pH 1.2, 1.4, and 1.5, complete inhibition was accomplished in 24 hr. Between pH 1.7 and 2.0, inhibition slowed down considerably, and at pH 2.1, no inhibition occurred. This experiment shows that the pH of a brine can be manipulated by brine formulation; i.e., the ratio of calcium hydroxide and calcium chloride in the sulfur dioxide solution.

It is to be pointed out that the pH value of the brine increases by 0.5 to 1.0 when the cherries enter the brine. This means that a brine with straight calcium chloride, or very little calcium hydroxide, may stay within the inhibition range when cherries are added. It is noted from experience *(12)*, however, that when a brine with a pH of 1.5 or lower is used, the cherries tend to crack and their skin injures easily. That is why some cherry briners refrain from using calcium chloride. It was further pointed out *(13)* that calcium chloride brine produces cherries with an undesirable, tough texture and low crispness, and the pits tend to stick to the flesh during the pitting operation.

Calcium chloride alone in water does not have any inhibitory effect on polygalacturonase other than producing a brine with lower pH.

Calcium hydroxide alone in water inhibits the enzyme completely as it produces an extremely high pH of 12.5.

A 1.25% sulfur dioxide solution alone inhibits polygalacturonase due to its low pH of 1.2.

Statistical analysis of the penetrometer readings revealed no significant difference between the effectiveness of various commercial alkyl aryl sulfonates as inhibitors of polygalacturonase. The only significant difference occurred in the sample without any alkyl aryl sulfonates.

Commercial alkyl aryl sulfonate preparations vary primarily in the amount of active ingredient present. Those with higher percentage of active alkyl aryl sulfonate can be used in proportionately lesser amounts.

While only 5 brined cherry samples with naturally-occurring pectic enzymes were obtainable, they furnished good evidence that natural enzymes in cherries are inhibited similarly to the commercial preparations, such as Pectinols.

These samples also show that the enzymes remain active in a cherry brine after 4 months. The brine shows a consistently higher enzyme activity than the cherries. This can be explained by the presence of inert solid materials in the cherries.

It is noted that the 0.6 ppm residual alkyl aryl sulfonate is found in the leached cherries which are not yet the finished products to be consumed. Any amount of handling during the manufacturing process of maraschino cherries will certainly reduce the residue further.

The fact that alkyl aryl sulfonate does not affect the finished maraschino cherries permits its regular use on cherries under any conditions. Since it is inexpensive, it can be used every time and becomes a part of the brine formulation. It serves as protection in the event these undesirable enzymes become prevalent again.

## SUMMARY AND CONCLUSION

Polygalacturonase has been found to be responsible for the severe softening of brined cherries by the degrading of pectin in the cherries. The enzyme is inactive at the pH of 1.6 or lower. By using calcium chloride instead of calcium hydroxide as the firming agent in $SO_2$ brine, such a low pH can be obtained. Nevertheless, at this low pH cherries tend to crack and their skin is injured easily. Calcium chloride brine also produces cherries with an undesirable, tough texture and low crispness. This is considered by many maraschino processors to be objectionable. For these reasons, calcium hydroxide is preferred as a firming agent in the cherry brine. The pH of a calcium hydroxide brine is about 2.1 and increases to about 3.0 when cherries are added. At this pH range, polygalacturonase remains active. The enzyme, however, can be inactivated by the addition of 0.01–0.025% of commercial alkyl aryl sulfonate to the brine. Five commercial preparations tested were found to be equally effective as inhibitors of polygalacturonase in $SO_2$–$Ca(OH)_2$ brine.

By use of radioactive istope tracer techniques, 0.6 ppm of residual alkyl aryl sulfonate was found to remain in the leached cherries when 0.01% of a commercial preparation of alkyl aryl sulfonate was added to the original brine.

No undesirable effects were observed by the authors on maraschino cherries made from brined cherries treated with as much as 0.1% of commercial alkyl aryl sulfonate.

It is concluded that polygalacturonase can be inhibited by a cherry brine with the following formulation: 1.25% sulfur dioxide, 0.7% calcium hydroxide, and 0.02% commercial 40%-active alkyl aryl sulfonate.

### Acknowledgment

The authors wish to express their appreciation to the various chemical companies who generously supplied chemicals used for this work. We are also grateful to Dr. C. H. Wang of the Chemistry Department, Oregon State College, for his help in radioactive isotope labeling and tracing of alkyl aryl sulfonate.

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# Effect of Packaging Films and Storage Temperatures on the Ripening of Mature Green Tomatoes[a, b]

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ONE FACTOR limiting production of tomatoes in Iowa and other northern states is a short growing season. To secure better yields and to prolong the time that the "home" crop is available, green tomatoes may be harvested for storage and ripening just prior to an expected killing frost. When tomatoes are shipped to northern states from the South, Southwest and Mexico, they are picked at the mature green stage; many of these fruits decay or ripen abnormally.

A number of years ago Haber *(4)* studied acidity and color changes in tomatoes held at various storage temperatures. Recently *(10)* Pentzer described faulty refrigeration practices during shipping and handling of tomatoes and other vegetables and fruits.

Within the last ten years methods of merchandizing mature green tomatoes have undergone considerable change. Many of these fruits are now handled in a central packaging plant where they are sorted on the basis of uniformity and maturity and are placed in cardboard or plastic trays overwrapped with cellophane transparent film. Packaging for self-service retailing is essentially a promotional device, but it does have the advantage of protecting the fruits from excessive bruising due to handling, thereby maintaining the tomatoes in better condition for a longer period of time. Since respiration and transpiration continue after harvest, a packaging material must be selected that will provide sufficient diffusivity to permit these processes to continue; otherwise, breakdown and fermentation will result. In the present study it was considered of interest to compare storage changes in tomatoes packaged in several commonly available transparent films with those occurring in unpackaged tomatoes.

## METHODS AND MATERIALS

Sioux, Rutgers, Big Boy, Manalucie and Pinkshipper tomatoes picked in an early stage of ripeness, but showing no yellow or red color, were harvested after the morning dew had evaporated from leaves and fruits. Some—or all—of the following criteria were used to assure that the fruits were mature green: (a) The parturition of the stem from the fruit (i.e., the absence of an open wound when stems were separated from the fruit, and the condition and color and the abscission zone of the fruit stems were felt to be indications of the stage of maturity). (b) If the skin of the fruits could be rubbed off easily, the tomatoes were considered immature since the skin of mature green tomatoes is well-formed, waxy and/or glossy. (c) From time to time, representative fruits were opened to determine if the method of selection was accurate. If the seeds were sufficiently hard to push aside and not be cut in two by a sharp knife and if there was a jelly-like substance in the locules, the tomatoes were considered mature-green. After the fruits were carefully sorted to remove all immature or poorly formed green fruits and fruits having growth cracks, sunscald, blemishes, insect bites, worm holes, bruises, softness and rot, each tomato was cleaned by gently washing in cold water and then dried with the aid of an electric fan. Before packaging, the fruit was sized and duplicate lots of four tomatoes each were placed in cardboard trays.[c]

Ripening was determined by the observation of color, firmness and acidity. To aid in evaluating maturity by color, a small judging booth was constructed and equipped with a 200-watt Sunshine Lighting Unit[d] (UCD-ADD-30). The illuminant provided a light intensity of 100 foot candles on the inspection table in the booth. Four judges assigned arbitrary values or growth "stages" ranging from 1–10 based on the following criteria:

(1) immature green (skin rubs off; open stem wound).
(2) mature green (well formed abscission layer, skin waxy and/or glossy. When cut, jelly-like locular substances and hardened seeds.)
(3) turning (slightly pink at blossom-end or streaks of pink, yellow or orange).
(4) half-pink, orange or yellow (>33⅓% but <66⅔% of surface colored).
(5) >66⅔% but <90% red (changing to red from pink, orange, yellow or green).
(6) >90% red
(7) completely red
(8) dead ripe (dark red in color but no softness or rot evident).
(9) over-ripe (dark, soft and/or evidence of rot spots).
(10) gross spoilage or rot manifest.

Firmness was determined subjectively by the use of slight hand pressure and objectively with the aid of a Lee-Kramer shear press. For shear press values, the tomato was cut in half from stem to blossom end in such manner that one of the locular walls was bisected while, on the opposite side, the locular contents were divided. Then the cut portion of the tomato was placed against the bottom grid of the sample chamber.

For the determination of pH, the fruit was triturated in a mortar and, after filtering the pulp through stainless wire screen to remove seeds, the pH of the juice was measured by means of a Beckman pH meter. Total acid was determined by titration according to the method of the Association of Official Agricultural Chemists *(1)* and the amount of acid present was calculated as citric acid.

A refractometer was used to determine amount of solids.

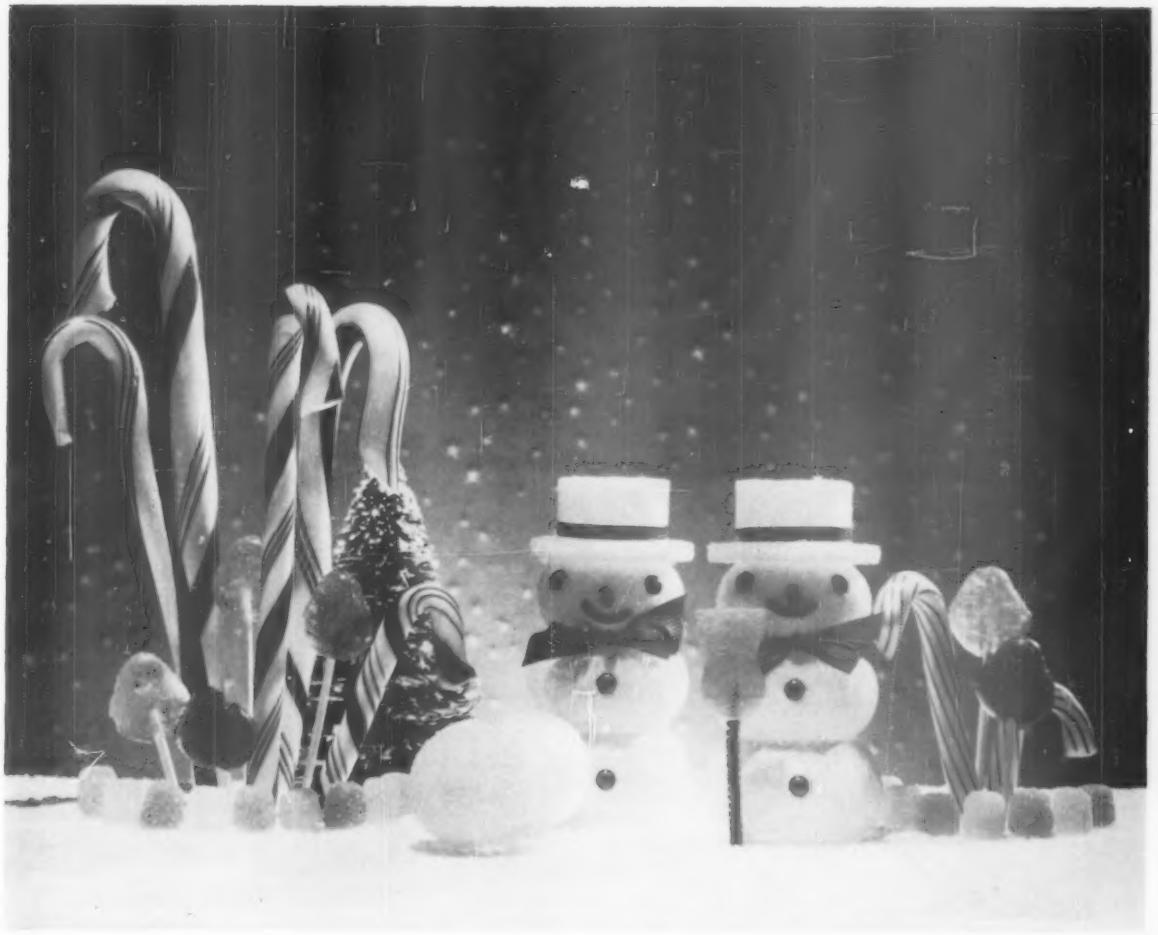
The tomatoes were stored in refrigerated display cases held at 5°, 10° and 15° C except for defrost periods. In addition, fruits were stored in rooms maintained at 20° C and at 25° C, and at ambient temperature. Tomatoes stored in the dark were held in wall-type refrigerators or in darkened rooms at the same temperatures as given above. Packaging materials used in-

[a] Journal Paper No. J-3868 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa, Project No. 1436.

[b] Eleven color slides were shown at the time of presentation. at the Twentieth Annual Meeting of IFT, San Francisco, California. Owing to the great cost of preparing color plates, these photographs could not be included. The authors will be pleased to loan 35 mm slides on a short-term basis to investigators interested in studying color changes.

[c] G-I green VU-More produce trays, Sutherland Paper Co., Kalamazoo, Michigan.

[d] Keese Engineering Co., Hollywood, California.



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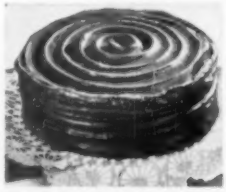

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cluded cellulose acetate (100 gauge), 300 LSAD and 300 PHD cellophane, Pliofilm (80 gauge), polystyrene Q641.4 (gauge 1), .0015 inch polyethylene, 517 Saran (gauge 50) and Cryovac (75 gauge).

Reflectance measurements for color using a Beckman spectrophotometer and a Gardner Color Difference Meter [commercial modification of the Hunter Color Difference Meter *(5)*] employed procedures similar to those by Robinson *et al (11)*, except that values for sections of cell walls rather than for tomato juice were measured. The cell wall tissue consisted of sections that separated the locular contents of adjacent cells. The surface of these cell walls was cut away leaving an area of approximately ⅜ inch thickness. Readings were made at 500, 520, 535, 545, 560, 600, 620, 645, 670 and 700 mμ. The tissue was placed in a 1½ inch cup (inside diameter) which was inserted in the sample drawer of the reflectance attachment. McCollum *(9)* and Robinson *et al (11)* caution that light may penetrate completely through the tissue and reflect from the background. However, since the same depth of sample and the same background were used for all tests, this source of error was minimized. The same sample was then tested in the Gardner Color Difference Meter for L, $a_L$, and $b_L$ readings.

## RESULTS AND DISCUSSION

Subjective tests of ripeness of the fruits on the basis of color, firmness, and taste were of only limited value. A consistent pattern of ripening at the various storage temperatures could not be observed. In several instances, tomatoes given a value of 2 by the judges during one test period were assigned a value of 6, 7, or 8 after a short period of storage; other tomatoes stored in the same tray at the same temperature still received values of 3, 4 or 5.

Even the most careful rubbing of the skin to separate immature green from mature green tomatoes resulted in skin breaks, scuff marks, bruises, or soft or dark areas that appeared later during storage. For this reason, evaluation of the state of maturity was usually restricted to visual observation. Similarly, it was considered that the application of slight hand compression for determining firmness likewise shortened storage life of the ripe fruit. Kattan *(6)* reported obtaining objective results with a measuring device called a Firm-o-meter but this device was not used in these preliminary studies.

When shear press readings were compared to values assigned by judges for changes in color of ripening tomatoes, it was found (Figure 1) that there was little texture change in tomatoes considered to be ±50%–, >66⅔%–, >90%– or completely red. This finding was substantially in agreement with the amount of time that elapsed for the fruits to proceed from stage 2 to 3 or from 3 and 4 to 7. The amount of time required for tomatoes to change from stage 3 to 7 often was no greater than from stage 2 to 3 or 7 to 8. This may indicate the desirability of using shear press values in determining ripeness.

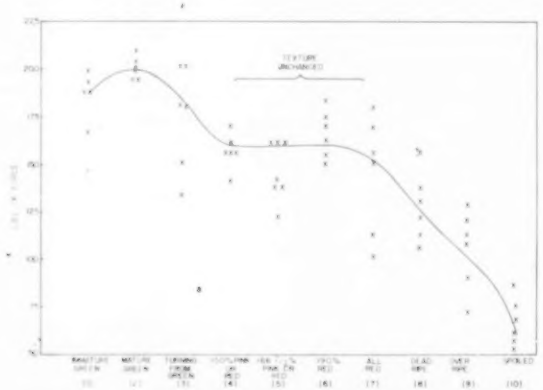

Figure 1. Relation of shear-press values to color of ripening tomatoes.

Judges usually were able to separate tomatoes in stages 1 and 2 on the basis of taste and generally were successful in distinguishing stages 3–6 from stage 8. However, there was some question as to the influence that the "mouth feel" of tomatoes had in determining judgment since the firmness of tomatoes in stage 3 or 4 differed appreciably from those in stage 8. Also, when the judges were blindfolded, separation of fruits assigned to stages 2 through 7 was much more erratic than if appraisal by appearance could be made at the same time. Often the comment for tomatoes that were almost—or completely—red was that these fruits were sweeter than those showing green or intermediate coloration. The judges expressed some dislike for tomatoes considered to be in stage 8 (dead ripe), usually indicating that these fruits were mealy, mushy, medicinal, flat, flat-sour, or bitter. The term *bitter* was used in describing tomatoes that had been stored at 5° or 10° C and/or overwrapped with Pliofilm, polyethylene, or Saran. Tomatoes classified in stages 9 and 10 were not given to the judges for taste.

The amount of water that the plant received either from recent rainfall or from abundant soil moisture was found to cause greater variation in the amount of total acid than did the variety of tomato used. These limited findings agree with the much more extensive trials made by Lee and Sayre in 1946 *(8)*. Likewise, it was observed that the amount of acid in fruits produced late in the season was less than that at the beginning of the crop year but no attempt was made to determine the influence of soil moisture during these periods. Acid values were calculated on a wet weight basis and per cent solids were checked using a refractometer. The amount of acid in green mature fruits was much higher than in ripe tomatoes. Extreme acid ranges varied from .18–.47 with an average value of .42 for tomatoes picked in August and .35 for tomatoes harvested in September. In contrast to these results, pH values showed only minor variations, with the usual extremes ranging between pH 4.0–4.4 except for fruits classified in groups 8, 9 or 10. With these last groups, it was observed that with an increase in pH value (and especially where pH >5.0), there was excessive softness, darkening, or appearance of rot spots.

Apparently storage life was determined to a greater extent by the percentage of total solids rather than by the variety of fruit. Tomatoes that had been harvested following a long wet spell had a solids content of approximately 6%, whereas those that were harvested during dry weather had a solids content of 6.7%. These latter tomatoes had an increased storage life of 3 weeks at 5° C and 10 days at 10° C over those that contained more water.

Coloration was found to be influenced by the environmental temperature. Tomatoes kept at cold temperatures did not have good external color development (see Figure 2) nor did the color within the tomato keep pace with that externally.

Even when kept for a period of 2½ months, tomatoes stored at 5° C failed to develop full color. When some of these fruits were removed after one month at 5° C and stored at ambient room temperature, many became watery and decayed rapidly without ripening. During storage the tomatoes had a mottled appearance and often the stems and points where these fruits were in contact with the tray became infected with mold. Flesh of most of the tomatoes was flaccid or limp. The decidedly pale pinkish-yellow cast of the tomatoes that did partially ripen might be explained by Denisen's finding *(3)*

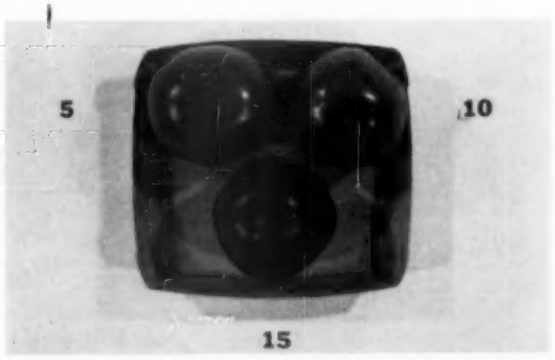


**Figure 2. Development of color in unwrapped tomatoes held for 14 days at 5°, 10°, and 15° C.**

that carotene formation is not inhibited by extreme temperature to the same extent as is lycopene.

Tomatoes held in the refrigerator at 5° C or at 10° C during the entire ripening period often appeared more blocky than fruits held at the same or higher temperatures in lighted display cases (Figure 3). In many of these fruits the fleshy locular wall separated from the seeds to provide internal "puffiness" or cavities in the seed chambers.

At 10° C, tomatoes had maximal satisfactory storage life. The fruits ripened more slowly than at a higher temperature and could be held in sound condition for a longer period of time. Generally, the time required for mature green tomatoes to develop uniform redness at 10° C was in excess of a month. At this temperature the ripe fruit remained in good condition for 35–45 days after color developed and often fruits did not show signs of spoilage for 2½ months. When these fruits were placed at room temperature, they remained firm for only 5–6 days. Color development was somewhat lighter than that of vine-ripened fruits and generally was more pink than red in hue. Also, color at 10° C was not as intense as at 20° C. In the display case in which the maximal temperature did not exceed 15° C and the minimal temperature of storage was held at 10° C, it was possible to keep tomatoes for a period of 70 days.

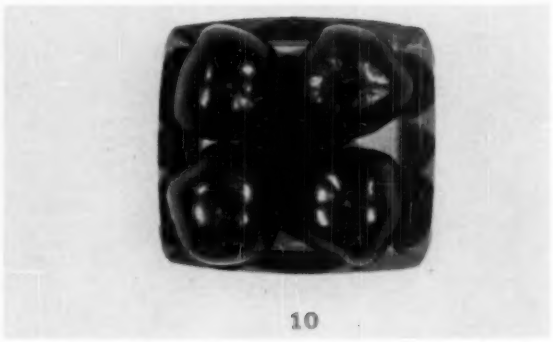


**Figure 3. Effect of low temperature on tomato contour.**

Fruits, either unwrapped tomatoes or those packaged in cellulose acetate, stored at 15° C ripened in a shorter period of time than at the two lower temperatures; the color of these fruits was found to be more intense (i.e., their L readings were greater). Seldom did these tomatoes keep for periods longer than 2 months and, usually, they reached the dead ripe stage of maturity within a one-month period. Many of the fruits remained sound for an additional 2–4 weeks at this temperature.

It should be pointed out that the 5°, 10° and 15° C temperature levels used in these studies refer to holding temperatures in refrigerated display cases, unless otherwise stated. Tomatoes stored at 10° C in refrigerators in the dark failed to develop color to the same extent as did those exposed to the fluorescent light in a refrigerated display case. Commonly the coils of these units are defrosted two or more times during each 24-hr period. During these defrost periods the temperature of the produce rises slightly (the total temperature differential being dependent upon the surrounding temperature and humidity, the amount of frozen condensate on the coils, and the total amount of produce in the storage chamber). Fruits stored in the dark were placed in a refrigerator having fairly constant temperature ($10° \pm 2 - 3°$ C) and humidity while those exposed in the display case were subjected to two 90-min cycle breaks in each 24-hr period during which their immediate environment changed by as much as 5–10° C. It is not known if these short exposures to increased temperatures may have accelerated ripening.

Tomatoes stored at 20° C colored more rapidly than at lower temperatures but spoiled in less than 1½ months. Generally the fruits ripened within 7–10 days and were dead ripe by 2 weeks. The tomatoes ordinarily remained sound for an additional week but, with longer storage time, showed signs of shriveling. Storage at 25° C resulted in still shorter shelf life than did 20° C. The tomatoes proceeded through the ripening stages within a period of a few days, and satisfactory storage for longer than one month at this temperature was exceptional. Even poorer storage life was obtained with ambient room temperatures. This was expected since temperatures prevailing were often >30° C and seldom did the temperature of the holding room fall below 22° C.

In Figure 4, $a_L$ and $b_L$ coordinates obtained from readings using a Gardner Color Difference Meter are plotted for tomatoes in stages of maturity ranging from mature green to dead ripe. Note that the points of intersection for curves (straight dotted lines) of outer- and of section-walls of ripe tomatoes coincide with values (straight broken lines) extrapolated from data supplied by Kramer *(7)* for color of Grade A, Grade C and Substandard tomato juice. Also, as might be expected, values for the stage at which tomatoes were considered ripe by visual observations intersected with values reported by Kramer *(7)* for Grade A tomato juice. Yeatmen *et al (12)* recently have pointed out some discrepancies between judges' values and $a_L/b_L$ ratios unless the position of the samples in 3-dimension color space was considered. They suggested that tomato color be determined in accordance with the formula:

$$2000 \frac{a_L}{\sqrt{a_L^2 + b_L^2}} \over L$$

In this same figure (Figure 4), spectrophotometric measurements of mature green and of ripe tomatoes are shown for comparison. It may be seen from color difference readings that measurements at the skin surface give values ranging further into the yellow and orange regions of the spectrum than do those of the cut section.

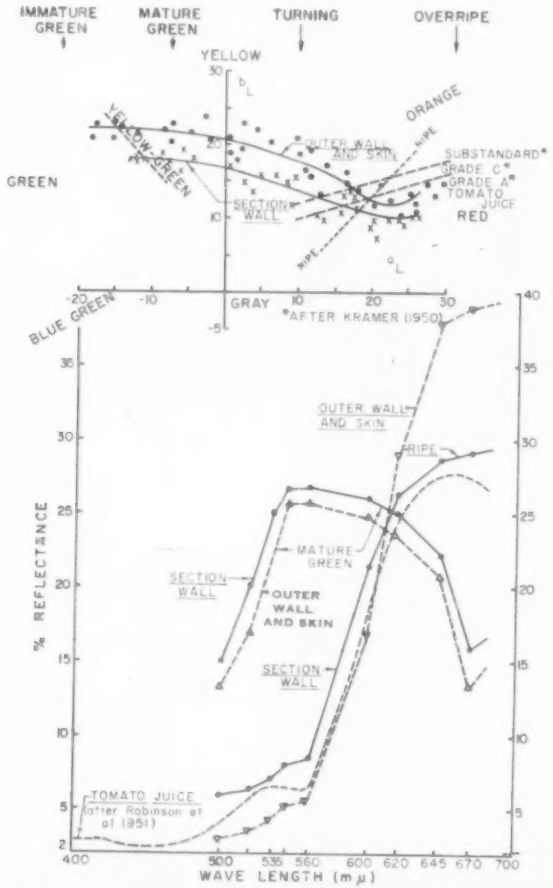


**Figure 4. Changes in $a_L/b_L$ readings and in reflectance values of tomatoes during ripening.**

Reflectance values for skin surfaces and cut-sections of mature green tomatoes have maxima at 545 and minima at 670 mμ while, with ripe tomatoes, maximal readings are approached at 620 mμ or above. This observation is in agreement with results reported by Birth *et al (2)* who used a rephobiospect to record spectral transmission values for the internal color of whole tomatoes.

Weight losses of tomatoes wrapped in each of several packaging films are shown in Figure 5. Tomatoes

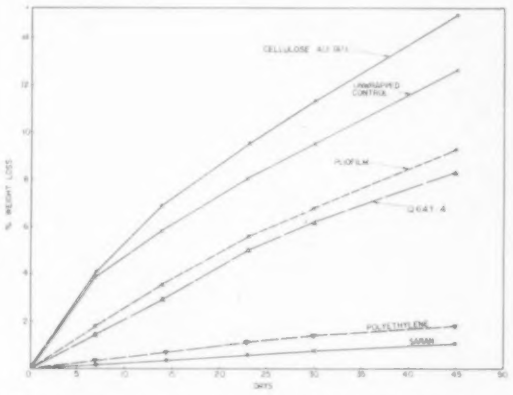


**Figure 5. Percent weight losses of tray-packaged tomatoes held at 15° C.**

packaged in cellulose acetate had greater loss of weight than those that were unwrapped. This might be explained by the loss of water vapor from the packages to the outside atmosphere during cycle breaks. Packaged tomatoes were more effectively insulated than were the unwrapped controls. As a consequence, these fruits did not cool as quickly and the moisture that did condense on the outside of the packages was lost by sublimation.

The following black and white photographic reproductions were made from color slides showing some of the more common changes that take place in the ripening of tomatoes when packaged with various packaging films. In Figure 6 it may be seen that, on the fourteenth day of storage at 15° C, tomatoes overwrapped with polystyrene and cellulose acetate presented excellent gloss. Also, these tomatoes exhibited slightly more intense coloration than did the unwrapped controls. The color of tomatoes placed in trays and overwrapped with polyethylene or with LSAD cellophane was somewhat less attractive. Tomatoes did not ripen normally when packaged with polyethylene; also, there was physiological breakdown of the fruits. The cardboard trays were wet and soggy and liquid often oozed from the tomatoes and was found to be free in the trays. In addition, there was an accumulation of moisture and fogging on the in-

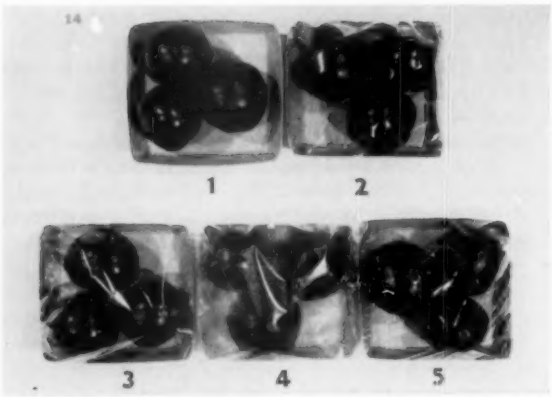


Figure 6. Unwrapped tomatoes (1) and tomatoes packaged in polystyrene (2), cellulose acetate (3), polyethylene (4), and cellophane (5) held for 14 days at 15° C.

side of the packaging film of the polyethylene-wrapped tomatoes. After storage for a period of 42 days at 15° C, the tomatoes in Figure 7 show even greater differences. Fruits packaged in cellulose acetate and plain cellophane were found to have higher redness values than similar fruits wrapped in any of the other films. Tomatoes stored at 10° C (Figure 8) packaged in Cryovac, polyethylene or Saran failed to develop desired red color whereas those placed in cellulose acetate, Q641.4 polystyrene or PHD cellophane had $a_L/b_L$ ratios of 1:2.1, 1:2.1 and 1:2.0 respectively. In addition to fogging of the tomatoes in the film, cardboard trays overwrapped with polyethylene and Cryovac became limp and water-logged during storage (see Figure 9). Similar changes were observed when fruits were overwrapped with Pliofilm or Saran. When tomatoes held in trays overwrapped with these films were removed from the trays, points of contact between the tomato tissue and cardboard showed evidence of rot; also there was mold rot at the stem scar in all of these fruits. Fruits that were considered to be turning at the time of packaging in cellulose acetate or polyethylene quickly developed rot at the blossom end when stored at 10° C.

In addition to abnormal coloring, tomatoes packaged with polyethylene caused the tray to warp owing to the high permeability of the film to respiration gases and in particular to carbon dioxide. This film failed to provide satisfactory control of gases. During the early part of the storage period, polyethylene overwraps were distended; later, after oxygen and carbon dioxide had migrated through the film, respiration demand resulted in reduced partial pressure within the tray.

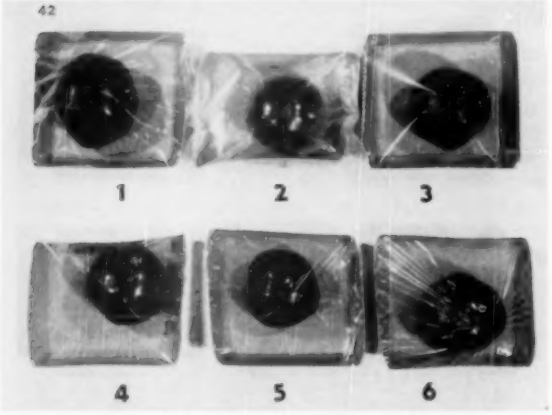


Figure 7. Tomatoes packaged in cellulose acetate (1), polyethylene (2), polystyrene (3), Saran (4), PHD cellophane (5), and Pliofilm (6) after storage at 15° C for 42 days.

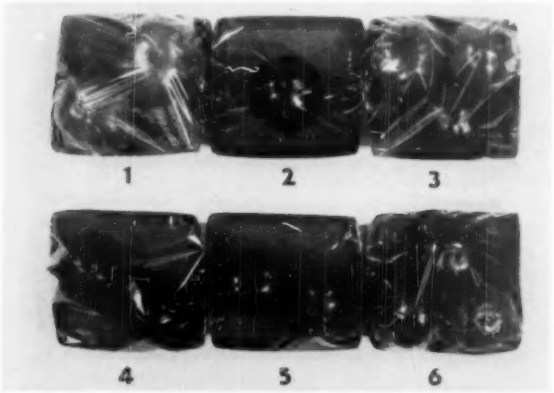


Figure 8. Tomatoes packaged in Cryovac (1), cellulose acetate (2), polyethylene (3), polystyrene (4), PHD cellophane (5) and Saran (6) after storage at 10° C for 63 days.

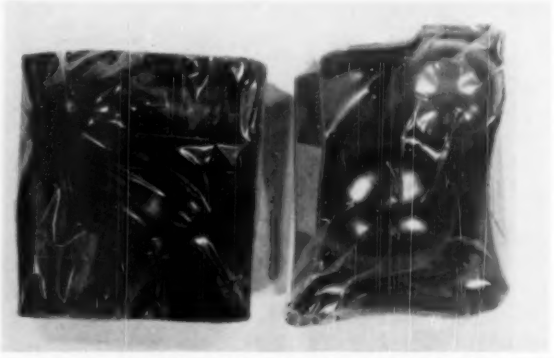

Figure 9. Condition of tomatoes, film, and packaging trays after storage in Cryovac and polyethylene at 10° C for 42 days.

## SUMMARY

Tomatoes stored at 5° C did not ripen normally; apparently this temperature was subminimal and predisposed the fruit to decay. At the other temperatures used, rate of ripening proceeded in the following order: ambient room >25° >20° >15° >10° C. Tomatoes kept for as long as 2½ months at 10° C and for 2 months at 15° C. The fruits spoiled in less than 1½ months at 20° C and in a few days at 25° C or at ambient temperature.

Use of shear press values appears to be of value in assessing stage of maturity (ripeness).

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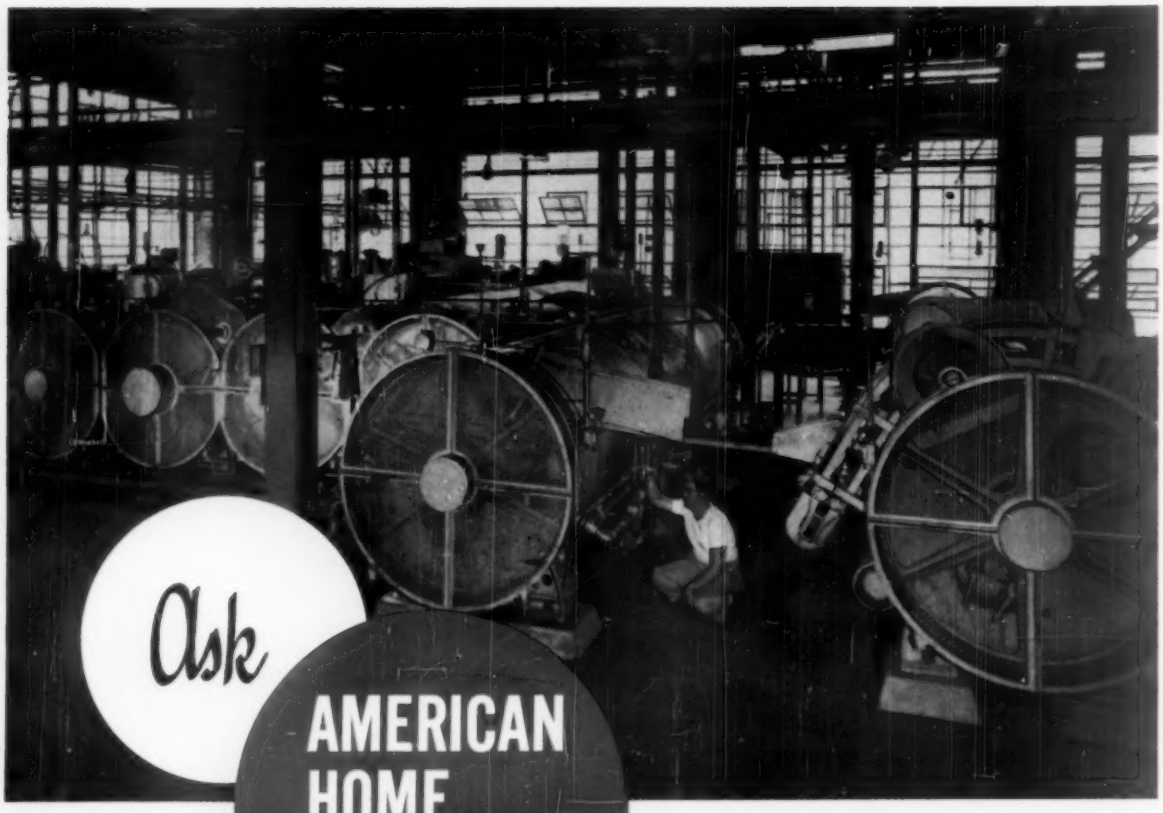

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Judging panels considered trays of tomatoes overwrapped with cellulose acetate (CA) attractive when compared with unwrapped tomatoes or with fruits packaged with polystyrene (Q641.4), polyethylene, PHD cellophane, Pliofilm, or polyvinylchloride (Saran) and Cryovac. CA had good transparency, adequate mechanical strength and sufficient permeability to gases and water vapor transmission. However, this film permitted greatest loss in weight of the product. There was some fogging in all of the other films; this defect was most obvious in trays overwrapped with the more impermeable types of packaging films. Not only did fruits rot more quickly in polyethylene, Pliofilm, Saran and Cryovac, but in addition, the tomatoes had less desirable taste. Weight losses were minimal in trays wrapped in Pliofilm, Saran and Cryovac but the fruit decayed and the trays became water-logged. Tomatoes did not ripen or color normally when packaged with polyethylene.

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# Instant Bread Mix: Studies on the Dehydration of Flavoring Materials*


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Department of Food Science and Technology, University of California, Davis


(Manuscript received May 16, 1960)

Development of an instant bread mix, using chemical leavening agents, which resulted in a product similar in physical characteristics to yeast-leavened bread, has been reported by Miller *et al (5)* of the Quartermaster Food and Container Institute for the Armed Forces, Chicago. This product was lacking in a major characteristic, flavor. Subsequent studies were undertaken by the same research group to determine the factors which influence the development of bread flavor precursors in a yeast fermented media *(1)*. These investigators proposed a formula and procedure for producing a fermented broth, which, when combined with the instant mix, resulted in a baked product with good sensory characteristics.

In order to fulfill the Armed Forces requirement for a complete mix, the next step in the developmental stages was naturally directed toward the production of a dry flavoring component. Limited work in this area was reported by Matz *et al (4)*.

This paper will discuss the application of three drying principles—freeze, spray, and drum—to the aforementioned fermented broth and the evaluation of these processes in the production of a dried flavoring medium from the standpoint of bread flavor.

* Presented at the Twentieth Annual Meeting of the Institute of Food Technologists, May 16, 1960, San Francisco, California.

## EXPERIMENTAL PROCEDURES

**Flavor broths:** Broth formulas used in the investigations (Table 1) were basically the same as those reported by Matz *et al (4)*. Fermentations were conducted at 37° C (± 2°) in 5 or 10 gal quantities, depending on the requirements of the specific drying procedures.

Removal of the yeast cells was accomplished with a Sharples supercentrifuge [b] at 26,000 rpm. It was necessary to centrifuge the broths twice in order to obtain a practical 0 cell-count.

**Freeze drying.** The cell-free liquid broths were frozen at −14° C in shallow pans, then dried in a conventional Stokes [c] freeze dryer. Both 1X and 3X broths were freeze dried with and without the addition of sucrose or flour. Flour was added in concentrations ranging from 5 to 50% on a weight basis.

**Spray drying.** Spray drying was conducted in a 15 x 25 ft conical spray dryer with a double reverse basket nozzle driven by a 25 hp electric motor at a speed of 30,000 rpm. Feed rate and input air temperature were varied in the individual experiments. The dried material was separated from the air stream by a small cyclone separator. Broths were dried with and without the addition of various concentrations of flour and dried lactose.

TABLE 1

Basic flavor broth formulas

| Ingredients | Broth Codes 1X | 3X |
|---|---|---|
| 0.1 M dibasic sodium phosphate, ml | 1000 | 1000 |
| Sucrose, g | 100 | 300 |
| Nonfat dry milk, g | 50 | 150 |
| Compressed yeast (Saccharomyces cerevisiae), g | 50 | 150 |

**Drum drying.** A pilot size unit with a drum approximately 2 ft in diameter and 2.5 ft in length was used. The drum was aluminum plated on its peripheral surface to prevent sticking and the unit was top-fed by means of an automatic feed mechanism. In the series of trials conducted, both the 1X and 3X cell-free broths were used. The various adjuncts to the broth before drying included methyl cellulose, dehydrogenated fat, flour, dry milk solids, corn syrup, and antifoam.

**Basic bread formulas and baking procedures.** Throughout the studies outlined above, the formula used in the preparation of the bread for the evaluation of the drying procedures was based on that developed by Miller *et al (5)*. This formula and the standard Army bread formula are included in Table 2. Because of the necessary addition of various components to the broth preparations to obtain suitable dried products, the basic instant bread mixtures with which the various dried products were incorporated were altered accordingly.

Procedures used for preparation of both yeast leavened and instant chemically leavened bread followed those reported previously *(5)*. Dough was prepared for two 500-g loaves for each baking trial.

For the instant breads, the dried mix and dried broth preparations were blended in a ½ HP Reco mixer [d]; water or liquid broth was then added and mixed at slow speed for 1 min and medium speed for 7 min. The dough was weighed into 500-g loaves, automatically moulded with an Acme Rol-Sheeter [e] into loaves approximately 2½ x 7½ inches, proofed at 25° C for 5 min, and baked in an electrically heated vertically rotating oven at 218° C for 25 min. The entire procedure required approximately 40 min.

Preparation of the standard Army bread (yeast-leavened) including mixing all dry ingredients in the mixer, dissolving the compressed yeast in a portion of the absorption water, and adding the yeast solution and water to the dry mixture. The dough was mixed at low speed for 1 min and medium speed for 7 min. Each 500-g aliquot was allowed to ferment for 2 hr, then punched. After 20 min the loaves were moulded and proofed for 60 min at 38° C and approximately 90% RH, and baked at 218° C for 30 min. All samples were cooled at room temperature overnight before subsequent evaluation the following day.

[b] The Sharples Corporation, Philadelphia, Pennsylvania.
[c] F. J. Stokes Machine Co., Philadelphia, Pennsylvania.
[d] Reynolds Electric Co., River Grove, Illinois.
[e] D. R. McClain & Son, Pico, California.

TABLE 2

Instant mix and standard Army bread formulas

| Ingredients | % of flour weight: Instant mix | Standard Army bread |
|---|---|---|
| Flour | 100.0 | 100.0 |
| Sucrose | 5.5 | 5.5 |
| Nonfat dry milk | 6.0 | 6.0 |
| Salt | 1.0 | 1.0 |
| Soda | 3.5 | ..... |
| Shortening | 5.0 | 5.0 |
| Glucona delta lactone (with 5% calcium stearate) | 9.0 | ..... |
| Yeast or yeast flavor solids [1] | 6.0 | 2.5 |
| Water or flavor broth | 57.0 (water) [2]<br>63.0 (broth) [3] | 65.00 |

[1] Yeast flavor solids as determined by refractometry of liquid flavor medium.
[2] When dried flavor preparations used.
[3] When liquid broth used.

**Sensory evaluation.** Twelve members of the Food Science and Technology Department volunteered to participate in the evaluation of the bread throughout the study. The training of the panel consisted of several weeks of evaluating flavor of bread produced by the yeast and chemically leavened procedures using various levels of liquid broth for flavoring. The 12 judges proved to be discriminating and consistent after the training period.

Experimental designs used included the complete block for studies of 4 or fewer samples and balanced incomplete blocks for studies of 5 or more. The judges evaluated no more than 4 samples at one session for "bread flavor" intensity and preference. The ranking technique was applied.

Data obtained from complete block designs were evaluated by the critical ratio method *(3)*. That from incomplete block designs was analyzed by the chi-square method of Durbin *(2)* when each judge did not complete the design, and by analyses of variance *(6)*, using judges average ranks as variates, when the judges completed the design.

**Sample presentation.** Bread samples were presented to the judges in random order on white plates coded with random 2-digit numbers. The samples were cut in cylindrical form of approximately 1½ inches in diameter from ½ inch slices. The 4 pieces of each sample presented to each judge were from slices taken from the same location in each loaf. Evaluation was conducted under low-red illumination in individual taste booths. The mechanics of tasting was left to the discretion of each individual panel member.

## RESULTS AND DISCUSSION

In the preliminary fermentation trials conducted in this laboratory, it was observed that before completion of the fermentation of the 3X formulation, curdling occurred when the broth reached a pH of 5.5 or below. The occurrence of curdling, which was most frequent when larger quantities than one gallon were fermented, was probably due to a combination of factors such as protein concentration, rapid rate of acid production, and temperature. Broths produced from the 1X formulation, ranging in pH from 6.3–6.6, never curdled. Thus, in subsequent fermentations of the 3X formulation, curdling was controlled by maintaining the fermenting medium above pH 5.5 with 1.0 N NaOH. The solids contents of the centrifuged broths obtained from the 1X and 3X formulations were 8%

± .5% and 21% ± 1%, respectively, throughout the studies reported here.

Results of panel evaluation of yeast-leavened and instant-mix breads of various flavor levels are included in Table 3. Flavoring in the instant-mix breads was added in the form of the centrifuged liquid media. The addition of a reduced sugar, glucose, and dry milk solids were added to compensate for the lower amount of flavor solids in the bread made with 1X broth as compared to that made with 3X broth. Data indicate that these additions, however, were ineffective in enhancing bread flavor, but did add sufficient sweetness to make the bread somewhat objectionable to the panel members. From this study, it was concluded that the difference in flavor level of bread made with 1X and 3X broths (a difference of 7% flavor solids) was not detectable by the panel. It is interesting to note that the instant mixes 1 and 3 were ranked slightly more (but not significantly) intense in bread flavor than the yeast-leavened bread. Preference for the yeast-leavened bread was probably attributable to textural characteristics, as the panel commented on this factor.

TABLE 3

**Bread flavor intensity and preference ranks of instant-mix and yeast-leavened breads**

**(Liquid broth flavoring)**

| Samples | Average ranks [1] | |
|---|---|---|
| | Flavor intensity [2] | Preference [3] |
| 1. Instant, 1X broth (6% flavor solids) | 1.79 | 1.96 |
| 2. Instant, 1X broth (6% flavor solids) plus 3% glucose and 2.5% dry milk solids | 2.20 | 2.29 |
| 3. Instant, 3X broth (13% flavor solids) | 1.80 | 1.96 |
| 4. Instant, no flavor | 2.40 | 2.45 |
| 5. Yeast leavened | 1.94 | 1.37 |

[1] Lowest rank = highest flavor intensity or preference; based on 54 evaluations/sample.
[2] Significant differences among samples at .05 probability level.
[3] Significant differences among samples at .001 probability level.

Preparations of 1X and 3X broths freeze dried well, but, due to the high concentration of invert sugars, it was impossible to remove the dried products from the drying pans before sufficient moisture was absorbed to make them sticky and impossible to handle. Trial batches using sucrose as a diluent of the invert sugars were unsuccessful because the broth was sufficiently active to reduce the sucrose.

Various concentrations of flour were added to act as a diluent and adsorptive agent which would prevent intimate contact between the glomerates of broth to produce a homogeneous dispersion but a non-homogeneous mixture. The hygroscopic factor was observed in dried broths with the addition of quantities of flour as high as 20%. Broth dried with flour at levels of 25, 30, 35 and 50% resulted in products which were too hard to be powdered for incorporation with the dry mix. Browning was also a factor in these dried products.

Trials similar to those conducted with the 3X broth were done with 1X broth. Sucrose, again, was unsuccessful in producing a stable dry product. However, the addition of flour in concentrations from 10 to 50% in all cases resulted in dried products which were light and powdery. No hygroscopicity or browning was observed after storage at room temperature for 6 months. The freeze dried broth with 15% flour was arbitrarily standardized as the product to be used for further investigations.

It was not possible to dry the 3X broth with or without the addition of flour under normal operating conditions of the spray dryer. Where it was possible to produce a dried product at a very high input temperature and low feed rate, the dried product was dark brown in color. At the lower input temperatures and higher feed rates the broth with flour added would not dry and stuck to the sides of the desiccator. All additional experiments were conducted with the 1X broth. Three broth mixtures—15% flour, 25% flour and 20% flour plus 9% lactose—were successfully spray dried into fine, white powders. The feed rates for production of these products using this particular spray dryer was approximately 300 lb per hr. The initial air input temperature averaged 170° C, and the output temperature of the dried stream was maintained at approximately 50° C by controlling the air rate. Some problems of dusting were encountered with the high concentrations of flour.

Results of a comparison of bread made with the freeze dried, spray dried, and liquid broths are included in Table 4. All of the samples contained 6% flavor solids on the per cent flour basis. Because the broth spray dried with 25% flour did not differ significantly from the freeze dried, this was set as a standard for future investigations. Broth dried with lactose was considered slightly off-flavored by several judges.

Preliminary trials of drum drying the 1X broth with the addition of 0, 15.5, and 25% flour did not yield satisfactory results. This broth without flour did not have sufficient solids to coat the drum, whereas the broths with the two levels of flour stuck and burned on the drums. Lowering the drum temperatures with concurrent reduction in drum rotational speeds did not alleviate the problems of sticking or burning.

Subsequent drum drying tests with the 3X broth, produced by controlling fermentations above pH 5.5,

TABLE 4

**Bread flavor intensity and preference ranks of instant-mix bread**

**(Liquid, freeze and spray dried flavorings)**

| Samples (6% flavor solids) | Average ranks [1] | |
|---|---|---|
| | Flavor intensity [2] | Preference [3] |
| 1. Freeze dried (15% flour) | 1.92 | 1.94 |
| 2. Spray dried (15% flour) | 2.33 | 2.31 |
| 3. Spray dried (25% flour) | 1.96 | 2.10 |
| 4. Spray dried (20% flour plus 9% lactose) | 2.02 | 2.01 |
| 5. Liquid, 1X | 1.76 | 1.63 |

[1] Lowest rank = highest flavor intensity or preference; based on 72 evaluations/sample.
[2] Significant differences among samples at .01 probability level.
[3] Significant differences among samples at .001 probability level.

included such adjuncts as: 1% methyl-cellulose, 1% and 5% dehydrogenated vegetable shortening, 10% flour, 1% non-fat dry milk, 11% corn syrup, 1% antifoam, and 10% flour plus 1.5% shortening. Of these trials, the mixture of flour and shortening yielded the most satisfactory product with respect to hygroscopicity and ability to flake finely enough for incorporation into the instant mix. The product was cream colored and slightly greasy in appearance. The other mixtures were discounted for one or more of several reasons: improper coating of drums, burning and scorching, or resultant hygroscopic dried product.

TABLE 5

**Characteristics of dried bread flavoring components**

| Method of drying | Broth | Broth adjuncts (wt basis) | Flavor solids in dried product | Appearance of dried product |
|---|---|---|---|---|
| Freeze | 1X | 15% flour | 35% | white, coarse powder |
| Spray | 1X | 25% flour | 25% | white, fine powder |
| Drum | 3X | 10% flour and 1.5% shortening | 66% | cream, flaky |

Panel evaluation of breads made with the most satisfactory product (Table 5) of each of the 3 processes resulted in a just significant difference in flavor intensity between the freeze and spray dried samples (Table 6). The drum dried sample was significantly lower in flavor intensity than the freeze dried, but no different from the spray dried. There was no significant preference among the three breads.

TABLE 6

**Bread flavor intensity and preference ranks of instant-mix bread**

**(Freeze, spray, and drum dried flavoring)**

| Samples (6% flavor solids level) | Average ranks [1] | |
|---|---|---|
| | Flavor intensity [2] | Preference [3] |
| 1. Freeze dried ( $\bar{c}$ 15% flour) | 1.67 | 1.93 |
| 2. Spray dried ( $\bar{c}$ 25% flour) | 2.15 | 1.93 |
| 3. Drum dried ( $\bar{c}$ 10% flour plus 1.5% shortening) | 2.19 | 2.15 |

[1] Lowest rank = highest flavor intensity or preference; based on 27 evaluations/sample.

[2] Significant differences between samples 1 and 2 and 1 and 3 at .05 and .01 probability levels, respectively.

[3] No significant differences.

These 3 preparations were held at room temperature under nitrogen for 2 months before large quantities of each were produced for subsequent consumer testing. There appeared to be no deterioration among the samples with regards to browning and moisture pick-up over that period of time. More thorough studies on the storage stability of these preparations, incorporated with the instant mix, would be desirable.

## SUMMARY

The applications of freeze, spray, and drum drying techniques were successful in producing dried bread-flavoring materials from cell-free liquid broths. Breads made with these mixtures were ranked slightly different in bread flavor intensity and no different in preference by a laboratory panel. These dried materials maintained sufficient storage stability, from the standpoint of observed appearance, color retention and moisture pick-up over a period of 2 months.

### Acknowledgments

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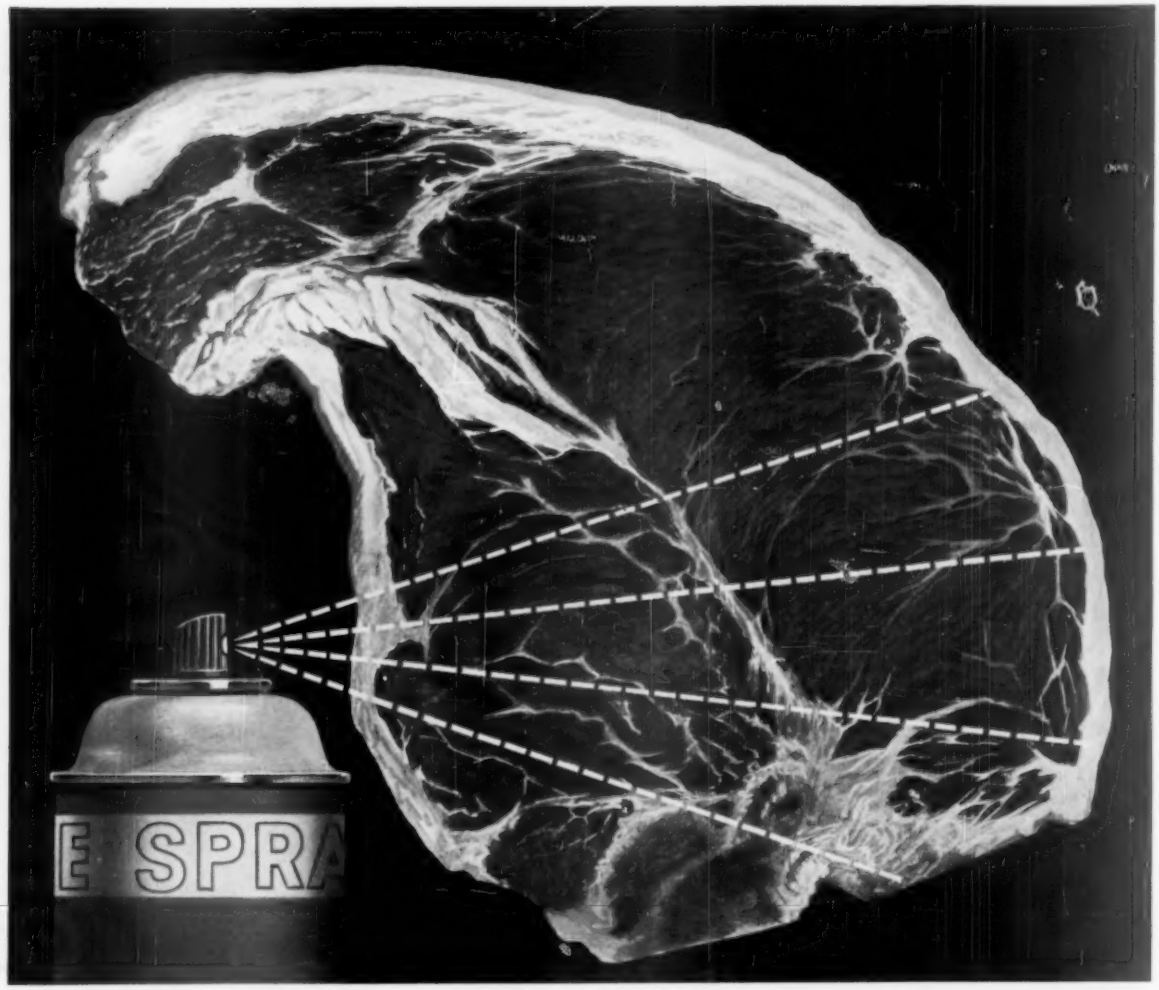


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# Instant Bread Mix: Consumer Evaluation of Prepared Bread[a]

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REQUIREMENTS of the Armed Forces for a bread formula which can be prepared with a minimum of time, equipment and skill, close to points of consumption, have been outlined by Matz *et al (5)*. An instant mix formula and a source of bread flavor components has been developed by these workers in the laboratories of the Quartermaster Food and Container Institute for the Armed Forces *(4, 5, 6)*. To completely satisfy the physical requirements as set forth, procedures to process the source of flavor components into a form which could be incorporated into the instant dry mix have been explored and developed by freeze, spray, and drum drying techniques *(2)*.

For this new type bread to be completely functional, it must be acceptable to the consumers from the standpoint of appearance, texture, and flavor. This is primary because the consumers for which it is being developed will not be influenced by the convenience factor, storage stability of the prepared bread, or factors other than those pertaining to eating quality.

This study was undertaken to determine the acceptability with regards to appearance, texture, and flavor of instant mix bread made with 4 flavor preparations. These factors were evaluated by a laboratory panel and a consumer panel of 100 households.

## EXPERIMENTAL PROCEDURE

**Bread.** Information on the development of the instant mix and fermented flavor media have been reported *(4, 5, 6)*. Processing techniques for the freeze, spray, and drum dried and liquid flavor preparations have been presented *(2)*. Quantities of each of these preparations were processed to flavor 300 loaves of instant bread. The dried flavors were stored in glass jars under $N_2$ in 5-loaf aliquots and stored at 0° C for use throughout the study. Liquid broth of the 1X formulation *(2)* was also weighed into jars and stored at −14° C. The compositions of these flavor preparations are listed in Table 1.

The addition of different amounts of flour or flour and fat necessary in the broth-drying processes *(2)* made it necessary to prepare different basic-mix formulas for each form of flavor medium. Composition of the resulting breads was in accord with the Quartermaster formula for instant bread with liquid broth *(5)*. Table 1 lists the bread-mix formulas used for each type of flavor medium.

Fifty-two 5-loaf batches of each of the 4 formulas were prepared following Army specifications, with the exception of the addition of the sodium bicarbonate and glucona delta lactone. These components were weighed separately into aliquots for each 5-loaf batch. This was done to alleviate any chemical reaction in the mix during the 8-week period of time the sensory evaluations were being conducted. The basic mix, sodium bicarbonate and lactone mixtures were stored in polyethylene bags at room temperature throughout the study.

The procedures for making the breads have been described *(2)*. Thirty 500 g loaves (six 5-loaf batches) of each of 2 samples were baked on each Tuesday and Thursday of the 7-week period of the study. After cooling for 4 hr, 1 loaf from each 5-loaf batch was measured for volume by the rape seed displacement method in a 1-lb. loaf volumeter.[b] The loaves were then sliced into ½ inch slices,[c] packaged in polyethylene bread bags and outer bakery paper bags and held overnight at room temperature for delivery to the consumers the following day (Wednesday or Friday). One loaf of each sample was also evaluated by the laboratory panel on each day after baking.

**Consumer panel.** Woodland, California, 12 miles from Davis, was selected as the source of consumers for the evaluation of the instant bread. A 2½% population sample (100 households of the 4000 in Woodland) was considered desirable for the study. Recruitment of the households was done by 2 methods, mail and telephone.

Using the telephone directory, 140 residents were selected randomly (approximately every 30th listing) and sent letters requesting their participation in the white-bread evaluation survey. No other information about the bread was made available to them. A card was enclosed for their response. The 46.6% favorable responses returned were not sufficient to fulfill the desired 100-household population sample. The lack of favorable response may have been due to the fact that Woodland was used previously for surveys of more acceptable or varied products such as wine, ice cream, and canned fruits.

Thus, 60 additional residents were selected and contacted directly by telephone. With approximately 73% favorable response by this direct contact, the combined 108 households were sent a second letter giving more detailed information of the survey procedure and asking information with regards to the number of household members 12 years of age or over and

[a] Presented at the Twentieth Annual Meeting of the Institute of Food Technologists, May 16, 1960, San Francisco, California.

[b] National Mfg. Co.

[c] Olivers Safety Slicer, No. 777.

TABLE 1

**Composition of flavor preparations and bread formulas**

| | Freeze dried | Spray dried | Drum dried | Liquid |
|---|---|---|---|---|
| Composition of broths: | | | | |
| Flavor solids, % | 35.4[1] | 25.4[1] | 65.7[2] | 8.5[1] |
| Flour, % | 64.8 | 74.6 | 29.8 | |
| Shortening, % | | | 4.5 | |
| Water, % | | | | 91.5 |
| Bread formulas[3]: | | | | |
| Flour, g | 1265 | 1182 | 1366 | 1400 |
| Nonfat dry milk, g | 84 | 84 | 84 | 84 |
| Sucrose, g | 77 | 77 | 77 | 77 |
| Shortening, g | 70 | 70 | 65 | 70 |
| Salt, g | 14 | 14 | 14 | 14 |
| Soda, g | 49 | 49 | 49 | 49 |
| Glucona delta lactone, g | 126 | 126 | 126 | 126 |
| Flavor preparations, g | 210 | 293 | 114 | 729 |
| Flavor solids, g | 75 | 75 | 75 | 75 |
| Flour, g | 135 | 218 | 34 | |
| Shortening, g | | | 5 | |
| Water, g | | | | 804 |
| Water, g | 804 | 804 | 804 | ..... |

[1] Calculated on basis of 8.5% solids in liquid broth by refractometry.
[2] Calculated on basis of 22.0% solids in liquid broth by refractometry.
[3] Quantities per 5–500 g loaf batch.

kind of bread eaten most frequently. They were also asked for their preference of the 2 delivery days and whether they would like to receive a summary of the results of the survey. Of the 108 second mailings, 100 responses were returned. These 100 households were subdivided into 4 groups of 25 each, based on delivery date and sample presentation.

The single stimulus method of sample presentation was used. In any one week the 4 samples of bread were evaluated; each sample by a different group of households. Groups I and II received delivery on Wednesday and Groups III and IV on Friday during a seven week period (Table 2).

**TABLE 2**

**Experimental design for bread evaluation**

| Week | Dates | Wednesday samples[1] | | Friday samples[1] | |
|---|---|---|---|---|---|
| | | Group I | Group II | Group III | Group IV |
| 1 | March 25–27 | A | B | C | D |
| 2 | April 1–3 | B | C | D | A |
| 3 | April 8–10 | C | D | A | B |
| 4 | April 15–17 | D | A | B | C |
| 5 | April 22–24 | B | D | A | C |
| 6 | April 29–May 1 | A | C | B | D |
| 7 | May 8–13 | D | A | C | B |

[1] Samples—A Freeze Dried Broth.
B Spray Dried Broth.
C Drum Dried Broth.
D Liquid Broth.

Consumers recorded their reaction to the samples on a 7-level structured Hedonic scale *(like very much* to *dislike very much)*. They were also asked to indicate if they liked or disliked the appearance, texture, and flavor of each sample, how each sample compared with commercial white bread and to write in additional comments. Participants indicated their sex and age group (12–18, 19–35, 36–50, and over 50) on their score cards. The score card was printed on unisort analysis cards. A card for each member was delivered with each sample along with an instruction sheet and an addressed, stamped envelope for returning the score cards.

**Laboratory panel.** A paired comparison evaluation of the instant bread samples was made in the Food Science and Technology laboratory simultaneously with the consumer evaluation. The 2 samples being evaluated by the consumers on Wednesday and Friday were also evaluated by the laboratory panel on those days (Table 2). The paired samples, served in random order, were evaluated by 12 judges experienced in bread tasting. Testing was conducted in individual taste booths. Each sample consisted of half a slice of bread from the same section in the sample loaves. Judges were asked to rank samples according to bread flavor intensity and preference. The laboratory panel evaluated each pair of samples twice, and completed its evaluation at the end of the 6th week.

**TABLE 3**

**Loaf volume measurement**

Average cc of displaced rape seed

| Replication[1] | Samples | | | | Replication average |
|---|---|---|---|---|---|
| | Freeze | Spray | Drum | Liquid | |
| 1 | 1830 | 1782 | 1700 | 2032 | 1836 |
| 2 | 1846 | 1797 | 1769 | 2051 | 1866 |
| 3 | 1917 | 1792 | 1736 | 2131 | 1894 |
| 4 | 1889 | 1804 | 1703 | 2077 | 1868 |
| 5 | 1857 | 1799 | 1737 | 2106 | 1875 |
| 6 | 1829 | 1774 | 1751 | 2048 | 1851 |
| Sample average | 1861 | 1791 | 1733 | 2074 | |

Least significant differences

| Probability level | Between sample averages | Between replication averages |
|---|---|---|
| .05 | 27 | 33 |
| .01 | 36 | .... |
| .001 | 46 | .... |

Analysis of variance

| Source of variation | DF | MS | Probability level |
|---|---|---|---|
| Samples | 3 | 667,029.65 | .001 |
| Replications | 5 | 7,993.38 | .05 |
| Samples × replications | 15 | 3,225.15 | ..... |
| Error | 96 | 2,714.48 | |

[1] Each replication of each sample is an average of volumes of 5 loaves.

**Analyses of data.** Laboratory panel evaluation results were analyzed by the chi square method *(7)*. Data obtained from the volume measurements and consumer panel ratings were analyzed by analysis of variance *(3, 7)*. The participants' general reactions were converted to numerical ratings; *like very much* = 7 to *dislike very much* = 1. Least significant differences were determined by the method of Alder and Roessler *(1)*. Significance of consumer responses to "liking" the three factors was determined by chi square.

## RESULTS AND DISCUSSION

Analysis of the volume measurements (Table 3) indicated a very highly significant difference among the 4 samples. Bread made with the liquid broth was largest in volume followed by freeze, spray, and drum dried samples. The slight significance in volume variation among replications cannot be explained.

In the laboratory panel evaluation (Table 4), the only comparison where significant differences in either bread flavor intensity or preference were noted was

**TABLE 4**

**Laboratory panel**
**Flavor intensity and preference**

| Pair compared | Bread flavor intensity | | | | | Preference | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number of judgments | Number of more flavor responses | | | | Number of judgments | Number of preference responses | | | |
| | | Freeze | Spray | Drum | Liquid | | Freeze | Spray | Drum | Liquid |
| A-B | 24 | 13 | 11 | | | 24 | 10 | 14 | | |
| A-C | 24 | 15 | | 9 | | 24 | 14 | | 10 | |
| A-D | 24 | 12 | | | 12 | 24 | 11 | | | 13 |
| B-C | 24 | | 15 | 9 | | 24 | | 11 | 13 | |
| B-D | 24 | | 7 | | 17 | 24 | | 7 | | 17 |
| C-D | 24 | | | 6 | 18* | 24 | | | 5 | 19** |

| Composite rank of samples—Flavor intensity | | | | Composite rank of samples—Preference | | | |
|---|---|---|---|---|---|---|---|
| Freeze dried | Spray dried | Drum dried | Liquid | Freeze dried | Spray dried | Drum dried | Liquid |
| 40<br>55.6% | 33<br>45.8% | 24<br>33.3% | 47<br>65.3% | 35<br>48.6% | 32<br>44.4% | 28<br>38.9% | 49<br>68.1% |

* .05 level of significance.
** .01 level of significance.

the comparison of the liquid vs. drum dried samples. A composite rating of the samples for both highest bread flavor intensity and preference resulted in the following rank order: liquid, freeze dried, spray dried, and drum dried. The relationship of the panel's response to flavor and preference with loaf volume can be seen in Figure 1.

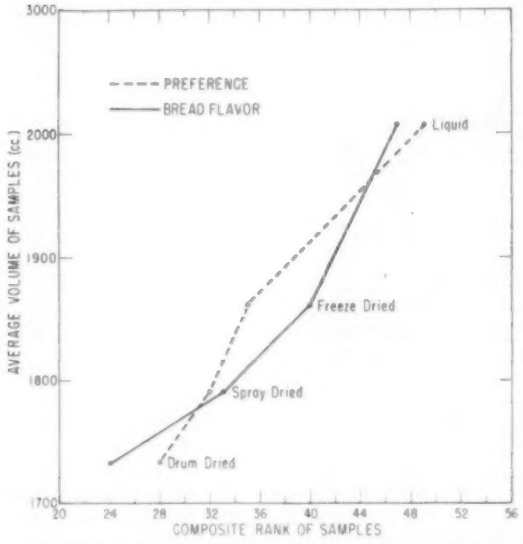


**Figure 1. Relationship of laboratory panel's response to flavor and preference with loaf volume.**

Data from the consumer survey represents responses from 94 households with an average of 2.7 persons in each. Six families, originally included in the survey, dropped out after the first 2 deliveries. Of the 254 score cards delivered each week, an average of 92.0% was returned. The highest returns were the first two deliveries with only slight variations in the following five. Distribution of age and sex among the 1,634 respondents is illustrated in Table 5.

**TABLE 5**

**Distribution of consumer responses by age and sex**

| Sex | Age group | | | | Total |
|---|---|---|---|---|---|
| | 12–18 | 19–35 | 36–50 | over 50 | |
| M | 168 | 213 | 232 | 212 | 825 |
| F | 124 | 189 | 333 | 163 | 809 |
| Total | 292 | 402 | 565 | 375 | 1,634 |

Effects of sample, age, sex and serving order on the consumer reactions are illustrated in Table 6. There was a significant difference in average ratings due to variation in flavor preparations among the breads. As with the laboratory panel, the liquid broth sample was rated highest. Although the consumers rated the dried broth samples in a different rank order than the laboratory panel, no significant differences

**TABLE 6**

**Effect of sample, age, sex and serving order on customers' over-all reaction**

Average ratings (7 = like very much to 1 = dislike very much)

| Samples | | Age | | Sex | | Serving order | |
|---|---|---|---|---|---|---|---|
| Freeze Dried | 4.53 | 12–18 | 4.98 | Male | 4.50 | 1st week | 4.61 |
| Spray Dried | 4.57 | 19–35 | 4.32 | Female | 4.88 | 2nd week | 4.66 |
| Drum Dried | 4.69 | 36–50 | 4.59 | | | 3rd week | 4.67 |
| Liquid | 4.84 | over 50 | 4.85 | | | 4th week | 4.67 |
| | | | | | | 5th week | 4.70 |
| | | | | | | 6th week | 4.71 |
| | | | | | | 7th week | 4.78 |

Least significant differences

| Probability level | Samples | Age |
|---|---|---|
| .05 | .24 | .24 |
| .01 | .... | .32 |
| .001 | .... | .41 |

Analysis of variance

| Source of variation | D.F. | M.S. | Probability level |
|---|---|---|---|
| Total | 223 | | |
| Samples | 3 | 1.5045 | .05 |
| Age | 3 | 4.7940 | .001 |
| Sex | 1 | 8.0106 | .001 |
| Serving order | 6 | 0.0869 | |
| Samples × age | 9 | 1.0389 | .05 |
| Samples × sex | 3 | 0.2250 | |
| Samples × serving order | 18 | 0.8071 | .05 |
| Age × sex | 3 | 5.6319 | .001 |
| Age × serving order | 18 | 0.5554 | |
| Sex × serving order | 6 | 0.0681 | |
| Error | 153 | 0.4373 | |
| Error (pooled)[1] | 180 | 0.4332 | |

[1] Used in determining significance as designated.

among these three samples were found by either panel.

The reaction of the participants to the instant bread, in general, fell between *like slightly* and *neither like nor dislike*, an average numerical rating of 4.65. Figure 2 illustrates the distribution of reactions by the consumers. Although approximately 64% of the responses were favorable (with 30% "liking moderately"), 31% of the reactions were on the *dislike* portion of the scale.

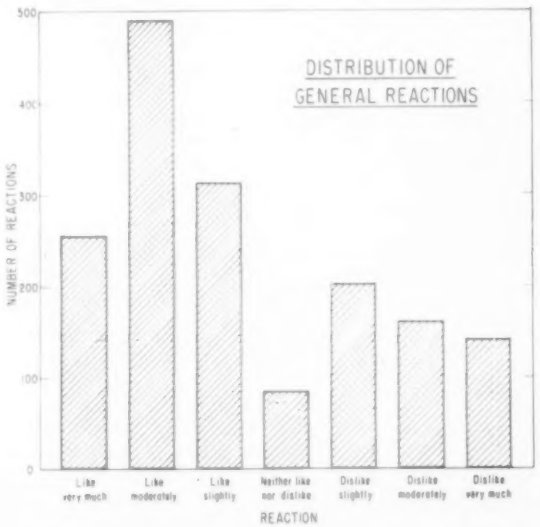


**Figure 2. Distribution of the 1634 general reactions by consumers to the four types of bread.**

The most significant factor in the analysis was the effect of sex, with the females rating the samples higher than the males. Age variation among the consumers was also a significant factor in their reaction to the instant bread, with the youngest and oldest groups rating the instant bread higher. Participants from 19 to 35, the age group encompassing the majority of men in the Armed Forces, liked the bread the least of any group. This group's average rating was 4.32.

There was no significant effect of serving order, although there was a consistent trend to rate the samples higher with each delivery. Lack of significance of the serving order effect is probably due to the significant interaction with samples, i.e. the freeze dried sample obtained its highest rating on the first serving, whereas the drum dried and liquid broth samples were rated highest on the seventh serving.

Respondents' reactions to "liking" the appearance, texture and flavor of each sample are illustrated in Table 7. It is apparent that the appearance of all the samples was the factor liked most by the consumers, followed by the texture factor. The only significant "like flavor" response by the population was with the liquid broth bread. Flavor responses appear to be more closely related to the overall ratings than either the appearance or texture factors. The parallel relationship of "like texture" responses to the loaf volume measurements is illustrated in Figure 3.

Consumer responses to comparative likeness of the instant bread and commercial sliced white bread (Table 8) indicate obvious preferences for commercial bread. The factors of sex and age affected a variation in response to this question, as they did in the rating of the samples.

The effects of the consumers' bread-eating habits on the average ratings and comparative likeness to commercial breads are indicated in Table 9. Of the households surveyed, the most frequently consumed bread was white sliced, followed by wheat, French, and homemade. Those who consumed French bread most frequently like the instant bread less than those who consumed the other types. The very small percentage of consumers (1.7 or 2 households) who ate homemade bread most frequently rated the samples very high (6.10, *like very much* to *like moderately*) and had a much lower "like less" response to the samples.

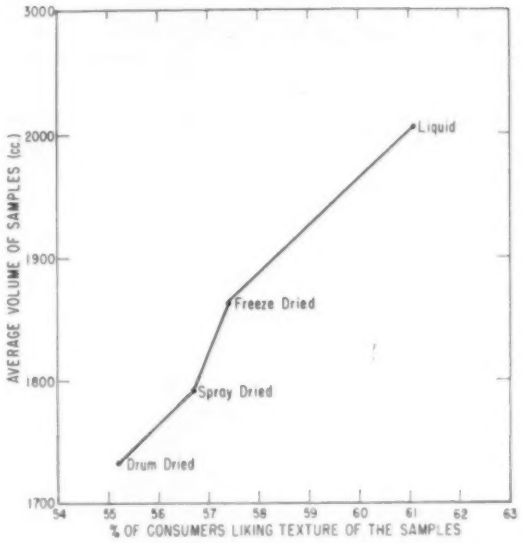


**Figure 3. Relationship between consumer 'like texture' response and loaf volume.**

**TABLE 7**

**Percent of population "liking" breads for various factors**

| | Sex | | Age group | | | | Combined total |
|---|---|---|---|---|---|---|---|
| | Male | Female | 12–18 | 19–35 | 36–50 | over 50 | |
| | % | % | % | % | % | % | % |
| Appearance | | | | | | | |
| Freeze Dried | 82.8[3] | 87.8[3] | 92.3[3] | 83.3[3] | 81.9[3] | 87.5[3] | 85.3[3] |
| Spray Dried | 79.1[3] | 86.5[3] | 80.3[3] | 81.7[3] | 82.5[3] | 87.1 | 82.1[3] |
| Drum Dried | 80.9[3] | 88.1[3] | 75.4[3] | 91.8[3] | 85.0[3] | 81.4[3] | 84.4[3] |
| Liquid | 85.8[3] | 90.4[3] | 88.7[3] | 86.8[3] | 87.2[3] | 90.9[3] | 88.1[3] |
| Texture | | | | | | | |
| Freeze Dried | 57.6 | 57.1 | 61.5 | 51.1 | 49.5 | 72.7[2] | 57.4[2] |
| Spray Dried | 54.6 | 58.8[1] | 55.9 | 47.1 | 57.3 | 66.3[2] | 56.7[1] |
| Drum Dried | 52.9 | 57.4 | 66.7[2] | 48.9 | 50.8 | 60.6 | 55.2 |
| Liquid | 60.3[2] | 61.8[2] | 63.1 | 48.4 | 59.7[1] | 78.1[3] | 61.1[2] |
| Flavor | | | | | | | |
| Freeze Dried | 42.0 | 48.5 | 59.6 | 38.0 | 37.8 | 53.9 | 45.2 |
| Spray Dried | 49.1 | 50.6 | 51.6 | 42.2 | 47.4 | 60.3 | 49.9 |
| Drum Dried | 47.5 | 51.4 | 53.2 | 51.6 | 41.6 | 56.8 | 49.4 |
| Liquid | 55.1 | 56.1 | 60.0 | 51.0 | 55.7 | 57.5 | 55.6[1] |

[1] .05 level of significance.
[2] .01 level of significance.
[3] .001 level of significance.

**TABLE 8**

**Percent of consumers liking instant bread better, same or less than commercial bread**

| Comparative likeness | Sex | | Age group | | | | Samples (broth) | | | | Total per cent |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | 12–18 | 19–35 | 36–50 | over 50 | Freeze | Spray | Drum | Liquid | |
| Better | 18.1 | 23.8 | 21.8 | 13.5 | 22.6 | 26.0 | 18.2 | 18.7 | 21.2 | 25.6 | 20.9 |
| Same | 21.3 | 22.1 | 25.3 | 18.6 | 20.9 | 23.0 | 21.4 | 20.7 | 21.4 | 23.1 | 21.7 |
| Less | 60.6 | 54.1 | 52.9 | 67.9 | 56.5 | 51.0 | 51.0 | 60.6 | 57.4 | 51.3 | 57.4 |

TABLE 9

**Effect of consumer bread eating habits on comparative preference and average rating of instant bread**

| Bread eating habits | Preference | | | | Average rating | |
|---|---|---|---|---|---|---|
| | Per cent of respondents[1] | Per cent liking instant bread | | | Per cent of respondents[2] | Rating[3] |
| | | Better | Same | Less | | |
| White sliced | 52.0 | 19.6 | 20.3 | 60.1 | 52.6 | 4.70 |
| French | 5.5 | 24.2 | 24.2 | 51.6 | 5.5 | 4.27 |
| Wheat | 10.1 | 33.9 | 18.9 | 47.2 | 9.8 | 4.95 |
| Homemade | 1.7 | 33.3 | 40.8 | 25.9 | 1.8 | 6.10 |
| Others | 30.6 | 17.6 | 21.6 | 60.8 | 30.4 | 4.46 |

[1] Based on 1574 responses.
[2] Based on 1634 responses.
[3] 7 = like very much to 1 = dislike very much.

TABLE 10

**Consumer comments on instant bread**

| | Per cent |
|---|---|
| Favorable Comments (Total no. 178) | |
| 1. Good toasted | 19.7 |
| 2. Good texture | 7.3 |
| 3. Better than commercial | 3.9 |
| 4. Good flavor | 3.3 |
| Others | 65.8 |
| Unfavorable Comments (Total no. 437) | |
| 1. Doughy, gummy, sticky | 16.7 |
| 2. Too dry | 11.2 |
| 3. Too heavy | 9.2 |
| 4. Flavorless or flat | 8.9 |
| 5. Too floury | 7.8 |
| 6. Texture too fine | 3.9 |
| Others | 42.3 |

Consumers who fell into the "others" group included those who ate a variety of bread types.

The percentages of consumers making various favorable and unfavorable comments are shown in Table 10. Of the total number of comments (615) there were more unfavorable than favorable. In general, the comments were directed more to texture than any other factor.

## SUMMARY

Instant breads, made with freeze dried, spray dried, drum dried, or liquid flavoring preparations, were evaluated by a laboratory and consumer panel. Comparative ratings of bread flavor and preference by the laboratory panel resulted in the following rank order of the samples: liquid, freeze dried, spray dried, and drum dried. The rank order of general likeness reaction by the consumer panel was liquid, drum dried, spray dried, and freeze dried. No significant differences were indicated by either panel among the three dried flavor preparations. An over-all consumer rating of the instant breads was 4.65 (between *like slightly* and *neither like nor dislike* on a 7-level structured scale).

There were significant age and sex effects on ratings of the instant bread by the consumer population tested. The younger and older groups and females, rated all samples higher than the middle age groups and males. Bread-eating habits had an apparent effect on the consumer's degree of liking of the instant bread.

The significant differences in loaf volume among the 4 samples were closely related to the laboratory panel's comparative ratings of flavor intensity and preference and to the consumer panel's response to textural quality.

## Acknowledgments

The authors are indebted to personnel at the Western Regional Research Laboratory, Albany, and Western Condensing Company, Petaluma, for their assistance in using their equipment for the drum and spray drying phases of this study, respectively. Thanks are also due the Red Star Yeast Company for graciously contributing the compressed yeast throughout the investigation. Funds for this work were furnished by the Quartermaster Food and Container Institute of the Armed Forces.

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# Notes and Letters

## Consistency Modification of Dairy Products[a]

PHOSPHATES AND CITRATES are used to modify and control the consistencies of milk-based products such as process cheese, instant pudding, and evaporated milk. It has been found that dicarboxylic acids such as adipic[b] affect the consistencies of milk protein dispersions. In systems where condensed phosphates have produced gels, adipic acid has reduced viscosity by as much as eightfold.

Various consistency modifications obtained in nonfat dry milk slurries with several reagents are shown in Table 1. The effects of trisodium acid pyrophosphate (TSAPP) and sodium ethylene-diamine-tetraacetate (EDTA), both complexing agents for calcium, show that the condensed phosphate increases viscosity to approximately 500 times that obtained with EDTA. Adipic acid alone forms acid curd. However, when 1%[c] adipic acid is added with EDTA or TSAPP, viscosity is reduced, and no curd is formed. With EDTA, the reduction is slight, but, with the phosphate, it is almost eightfold.

TABLE 1

**Effects of pyrophosphate, adipic acid, and ethylene-diamine-tetracetate on viscosity of 25% slurries of nonfat dry milk**

| Additive | Viscosity (Centipoise) | pH |
|---|---|---|
| Control | 20 | 6.3 |
| 3% trisodium acid pyrophosphate (TSAPP) | >16000 (brittle gel) | 6+ |
| 5% sodium ethylene-diamine-tetraacetate (EDTA) | 33 | 6+ |
| 3% TSAPP, 1% adipic acid | 2200 | 5.3 |
| 5% EDTA, 1% adipic acid | 31 | 5.3 |
| 3% TSAPP, HCl to pH | curd | 5.3 |
| 5% EDTA, HCl to pH | curd | 5.3 |

Adipic acid appeared less prone to curd formation than hydrochloric acid. This is shown in Table 1 where curd formed with hydrochloric acid in the presence of TSAPP or EDTA at the same pH in which no curd was observed with adipic and TSAPP or EDTA.

Some similar effects were noted in a ten per cent dispersion of sodium caseinate. Table 2 shows a slight increase in viscosity when adipic acid alone is used and a marked increase with condensed phosphate (TSAPP). Again, the high viscosity obtained with the phosphate is signficantly reduced by adipic acid. This reduction is not due to phosphate hydrolysis, since more than 30 days are required for 5% hydrolysis at pH 5.3 and 30° C *(1)* (in the absence of phosphatase activity which should be minimal after the heat treatment in the dry-milk process). It may be due to an interference with the characteristic phosphate-casein interaction.

TABLE 2

**Effects of adipic acid and pyrophosphate on viscosity of 10% dispersions of sodium caseinate**

| Additive | Viscosity (Centipoise) |
|---|---|
| Control | 31 |
| 3% TSAPP | 1250 |
| 1% adipic acid | 38 |
| 3% TSAPP, 1% adipic acid | 100 |

These effects are obtained at room temperature when the milk solids are added to solutions of the additives. Viscosities in Tables 1 and 2 were measured on a Haake Rotovisko viscometer. Similar results were obtained when materials were combined at 50° C.

Results suggest interesting possibilities in controlling consistencies of cheese products, cocktail dips, evaporated milk, dairy-based sauces, etc. Since the data were obtained with milk solids, it is probable that some such products could be marketed in the dry form.

A. R. HANDLEMAN
Research Department
Inorganic Chemicals Division
Monsanto Chemical Co.,
St. Louis, Missouri

[a] Contribution of Monsanto Chemical Company, Organic Chemicals Division.

[b] Adipic acid was chosen for this study, because it has been generally recognized as safe for food use.

[c] Additive percentages based on amount of milk solids rather than total. Milk solids percentages are by weight of total.

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662A

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Bulletin A-1 describes their advantages, includes a list of 69 pertinent scientific papers and patents, and a compilation of projected uses.

This information will be of interest to food processors in view of the recent official approval* of the use of these materials, up to 5% in finished foods, as a food coating agent, container component, equipment lubricant, or emulsifier.

*See Regulations of the Food & Drug Administration Sec. 121.1018

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662B

# A Note on the Application of Orthogonal Polynomials to Data on Palatability when the Levels of a Treatment Are Unequally Spaced

THE LEVELS of a treatment frequently are chosen by the experimenter to be equally spaced so that the method of orthogonal polynomials can be conveniently employed in determining the appropriate regression curve. The use of orthogonal polynomials with texture scores of broccoli cooked electronically for four equally spaced times is illustrated in *(3)*. The polynomials were constructed from recursion formulas which are based only on equally spaced values of X (treatment levels). When the levels of a treatment are unequally rather than equally spaced, the polynomials can be constructed by following the method proposed by Grandage *(5)*.

The purpose of this paper is to illustrate the use of orthogonal polynomials with palatability data when the treatment levels are unequally spaced. The polynomial expressions derived in *(5)* are applied in testing the fit of linear, quadratic and cubic regression curves in successive stages and in calculating the regression equation.

## METHODOLOGY

As for equally spaced X *(3)*, the regression equation in terms of orthogonal polynomials is

$$\hat{Y} = \overline{Y} + A_1P_1 + A_2P_2 + \ldots + A_rP_r \qquad \text{(Equation 1)}$$

where: $\hat{Y}$ is an estimated value of Y, $\overline{Y}$ is the mean of Y.

A's are functions of partial regression coefficients.

P's are orthogonal polynomials; $P_1$ is a function of X, $P_2$ of X and $X^2$, and so on.

Orthogonal polynomials have the following properties:

1. The sum of the values for each polynomial is zero.
2. The sum of the cross products of any two polynomials is zero.

The procedure illustrated in *(5)* for calculating the polynomial values when X is unequally spaced involves solving simultaneous equations formed by applying the properties of orthogonal polynomials.

The polynomial values and expressions for four unequally spaced X are shown in Table 1. It can easily be verified from the last three columns of the table that $P_1$, $P_2$ and $P_3$ are orthogonal polynomials: the sum of the values for each polynomial is zero and the sum of the cross products of two polynomials is zero. The latter property is illustrated by

$$\Sigma P_2P_3 = 7\,(-3) - 4\,(8) - 8\,(-6) + 5\,(1) = 0.$$

The polynomial values can be checked by substituting a value of X′ in any of the three expressions. For example, when X′ is 0, $P_1 = 4(0 - 7/4) = -7$, as shown in the first row of the fourth column.

## RESULTS AND DISCUSSION

Example. The data for this example are taken from the results of a taste experiment made on fresh broccoli cooked by boiling for four unequally spaced times: 5, 10, 15, 25 min *(4)*.

Orthogonal polynomials were employed in deriving the regression equation for estimating flavor scores of broccoli heads from various cooking times. The procedure is illustrated in the following steps:

Step 1: Let Y denote mean panel flavor score and X denote cooking time in the equation

$$\hat{Y} = \overline{Y} + A_1P_1 + \ldots + A_rP_r$$

Panel mean scores and the sum of squares for "between cooking times" and for each polynomial are included in Table 1. The sum of squares for $P_1$, for example, is

$$\Sigma P_1^2 = (-7)^2 + (-3)^2 + (1)^2 + (9)^2 = 140$$

The values of $A_1$, $A_2$ and $A_3$, also are included in the table. $A_1$, for example, is given by

$$A_1 = \frac{\Sigma P_1 Y}{\Sigma P_1^2} = \frac{-7(2.40) + (-3)(3.60) + 1(3.33) + 9(2.06)}{140} = \frac{-5.73}{140} = -.0409$$

Step 2: In the analysis of variance (Table 2) the variation for "between cooking times" is broken down into three independent regression components. Each component is successively tested against "error" to ascertain whether its addition in the regression equation reduces the variation by a significant amount. In this manner, the goodness of fit of each curve—linear, quadratic and cubic—is independently determined. Therefore, by using orthogonal polynomials, the fit of each regression curve is quickly and easily tested even when the values of X are unequally spaced.

TABLE 1

**Values of orthogonal polynominals and mean scores for flavor of broccoli**

| Cooking time X | X′ = X/5 − 1 | Flavor score[1] Ȳ | Polynomial values | | |
|---|---|---|---|---|---|
| | | | $P_1$[2] | $P_2$[3] | $P_3$[4] |
| *minutes* | | | | | |
| 5 | 0 | 2.40 | −7 | 7 | −3 |
| 10 | 1 | 3.60 | −3 | −4 | 8 |
| 15 | 2 | 3.33 | 1 | −8 | −6 |
| 25 | 4 | 2.06 | 9 | 5 | 1 |
| Sum | .... | 11.39 | 0 | 0 | 0 |
| $\overline{Y}$ | .... | 2.85 | .... | .... | .... |
| Sum of squares | .... | 34.0525 | 140 | 154 | 110 |
| $(\Sigma Y)^2/4$ | .... | 32.4330 | .... | .... | .... |
| Sum of squares "between cooking times" | .... | 1.6195 | .... | .... | .... |
| $A_i = \Sigma P_i Y/\Sigma P_i^2$ | .... | | −.0409 | −.0905 | .0335 |

Source: Values and expressions for the polynomials are taken from *(5)*.

[1] 1–5 scale on natural flavor: scores of 3–5 denoted moderately full to full and 1–2 weak to moderately weak *(4)*.

[2] $P_1 = 4\left(X' - \frac{7}{4}\right)$.

[3] $P_2 = \frac{7}{2}\left(X'^2 - \frac{29X'}{7} + 2\right)$

[4] $P_3 = \frac{55}{12}\left(X'^3 - \frac{63X'^2}{11} + \frac{392X'}{55} - \frac{36}{55}\right)$.

TABLE 2

**Analysis of variance: Breakdown of "between cooking times" into components of regression**

| Source of variation | Sum of squares[1] | Degrees of freedom | Mean square | F |
|---|---|---|---|---|
| Between cooking times | 1.6195 | 3 | 0.5398 | 7.8** |
| $A_1P_1$: Linear component | .2344 | 1 | .2344 | 3.4 |
| $A_2P_2$: Quadratic component | 1.2616 | 1 | 1.2616 | 18.2** |
| $A_3P_3$: Cubic component | .1233 | 1 | .1233 | 1.8 |
| Error[2] | .1667 | 24 | .0694 | |

** Significant, 1% level.

[1] The sum of squares for the three components equals, except for rounding, the sum of squares for "between cooking times." The sum of squares for each component is $A_i \Sigma P_i Y$.

Example: $A_1 \Sigma P_1 Y = (-.0409)(-5.73) = .2344$.

[2] Interaction of judge × cooking time × session furnished by a complete analysis of variance.

As shown by the F test, the quadratic component was the highest component, and incidentally the only one, that was significant. Therefore, a quadratic curve appropriately represents the regression of flavor score on cooking time of fresh broccoli.

Step 3: The regression equation of flavor score on cooking time is the quadratic

(Equation 2)

$$\hat{Y} = \overline{Y} + A_1P_1 + A_2P_2 = 2.85 - .0409P_1 - .0905P_2$$

since $\overline{Y} = 2.85$, $A_1 = -.0409$ and $A_2 = -.0905$ from Table 1.

From footnotes 2 and 3 of Table 1, we have

$$P_1 = 4X' - 7$$

$$P_2 = \frac{7}{2}\left(X'^2 - \frac{29X'}{7} + 2\right)$$

Since $X' = X/5 - 1$, then

$$P_1 = .8X - 11$$

$$P_2 = 3.5\left(\frac{X}{5} - 1\right)^2 - 14.5\left(\frac{X}{5} - 1\right) + 7$$

$$= .14X^2 - 4.3X + 25$$

Substituting these expressions in Equation 2, we have

$$-.0409P_1 = -.0409\ (.8X - 11) = -.033X + .450$$

$$-.0905P_2 = -.0905\ (.14X^2 - 4.3X + 25)$$

$$= -.013X^2 + .389X - 2.262$$

and therefore

(Equation 3)

$$\hat{Y} = 2.85 - .033X + .450 - .013X^2 + .389X - 2.262$$

$$= 1.04 + .356X - .013X^2 \qquad \text{(Equation 4)}$$

which is the quadratic regression equation of flavor score, Y, on cooking time, X.

Equation 3 clearly shows that the quadratic is formed by adding $-.013X^2 + .389X - 2.262$, the last three terms, to the linear equation, $\hat{Y} = 2.85 - .033X + .450$. Thus, even when the values of X are unequally spaced, a regression equation expressed in terms of orthogonal polynomials can easily be formed merely by adding additional terms to the linear equation.

Step 4: The curve (Figure 1) plotted from the quadratic equation shows that the estimated scores for flavor fluctuated with increasing cooking time, rising irregularly from 2.5 at 5 minutes to a peak of 3.5 at 13–15 minutes and then falling rapidly from 3.0 at 20 minutes to a low of 1.8 at 25 min. The cooking time of 13 min yielded broccoli of best texture *(2)*.

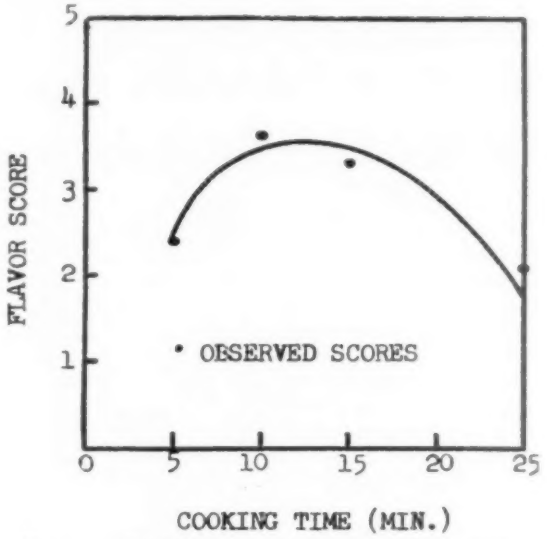


**Figure 1. Quadratic regression curve of flavor score on cooking time of fresh broccoli.**

## SUMMARY

Orthogonal polynomials are used to evaluate panel scores for flavor of fresh broccoli cooked by boiling for four unequally spaced times: 5, 10, 15, 25 minutes. A quadratic equation, $\hat{Y} = 1.04 + .356X - .013X^2$, is calculated for estimating flavor score from cooking time. This equation is formed by adding several terms to the linear equation. The example demonstrates that, even when the treatment levels (X) are unequally spaced, the goodness of fit of the linear, quadratic and cubic regression curves can be independently and successively tested for significance.

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July 1, 1960.

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## Errata, Food Technology, Volume 14 (1960)

In the non-technical pages of FOOD TECHNOLOGY 14 (3) 1960 the bibliographic entry on page 36 should read "Zucker und Zuckerwaren, Albrecht Fincke."

In the article by M. D. Berman, Determination of the proximate and electrolyte content of beef, FOOD TECHNOLOGY 14, 429 (1960) the first three lines of paragraph 3, page 430, should read: "*Determination of phosphorus.* Phosphorus present as the phosphate was determined by measurement of the molybdivanadophosphoric acid complex (9), which was stable for up to 2 days."

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The Professional Society of Food Technologists

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# Institute of Food Technologists

**The Institute of Food Technologists,** the professional society of Food Technologists, numbers among its active members leading scientists, technologists and engineers in the field of Food Technology.

**The Institute invites your professional association** with this select group of scientific and technological workers. Only those who are qualified by education, special training, or experience are extended this invitation.

*Food Technology may be defined as the application of science and engineering to the production, processing, packaging, distribution, preparation and utilization of foods.* Scientists, technologists and engineers engaged in Food Technology, executives, officials and supervisors under whose jurisdiction Food Technology operations are conducted, research investigators, teachers, and others working in the field of Food Technology, will find membership in the Institute worth many times its modest cost.

**If you can meet the qualifications for membership, you owe it to yourself and to your business or profession to become a member.**

## OBJECTIVES

The Institute is a non-profit, professional, educational association to promote the application of science and engineering to the production, processing, packaging, distribution, preparation, and utilization of foods; it stimulates investigations into technological problems arising therein, it provides a medium for presenting and discussing the results of such investigations; it stimulates and provides means for the free interchange of technological information and ideas among food technologists; it promotes recognition of the role of food technologists in the technical direction of operations in these fields; all of these activities have the ultimate objective of providing better and adequate foods for mankind.

## ORGANIZATION AND PROGRESS

Organized July 1, 1939, at Cambridge, Mass., with a membership of less than 100, the Institute has grown to more than 6,000 (1960). It is world-wide in scope, with members in North, Central, and South Americas, the Scandinavian countries, England, Holland, Germany, France, India, Australia, New Zealand, and Japan.

## MEMBERSHIPS

**Professional Members.** Any person who is ethically qualified, and who has had training and experience in food technology, or any person who in the opinion of the Council is recognized as distinguished in the contributing sciences as they apply to foods, shall be eligible to be a Professional Member of the Institute. The minimum training which shall qualify a candidate for eligibility for Professional Member is, in general, graduation from a college, university, or similar institution in which he has majored in one or more of the sciences or branches of engineering associated with food technology. The minimum experience shall be three years experience in food technology. The Council may, at its discretion, waive any or all of the foregoing requirements in the case of a person who, thru long experience has distinguished himself in the field of food technology.

**Members.** Any ethically qualified person, active in special or limited aspects of food technology, who is an administrator, director or executive under whose jurisdiction operations in food technology are conducted; or who is engaged in the dissemination of knowledge of food technology; or one whose work requires a general knowledge of the broad principles of food technology as it applies to the products, processes or equipment with which he or she is concerned; or one scientifically trained in and who has entered a career in food technology; shall be eligible to become a Member.

**Student Members.** Any ethically qualified person who has never been a Professional Member or Member of the Institute, who is registered as a student in an educational institution with at least Junior (third year) standing, who is a candidate for a Bachelor's or higher degree in one or more of the sciences or branches of engineering associated with food technology, shall be eligible for membership as a Student Member. This classification shall be his status only until the end of the calendar year in which he completes his schooling. When graduated and otherwise qualified as defined in the section on "Members," a Student Member may, upon application, be elected to Membership.

## DUES

Professional Members and Members—$10.00 a year; Students—$2.50 a year. Subscription to Food Technology included. Foreign postage: Central & South America—$1.00 additional; all other countries—$2.00 additional.

## PUBLICATIONS

Food Technology, issued monthly, is the official journal of the Institute. It is published to advance the profession of food technology by disseminating the results of research and their practical application in industry. Contributors to its pages represent many fields of scientific and technological interest.

Food Research, published bi-monthly, is devoted to publication of the results of original investigations and occasional reviews in the fields of food and beverage technology, chemistry, and microbiology. Food Research is available to Institute members at the special rate of $7.50 per year.

## REGIONAL SECTIONS

Where sufficient members (25 or more) live within commuting distance of a given point, a Regional Section may be established. Such Sections are able to hold meetings at more frequent intervals than the National group, thus affording the food technologists regular opportunities for an exchange of views and ideas with others in the area. There are presently 33 Regional Sections.

## ANNUAL MEETINGS

Each year one of the regional sections acts as host to the Institute at an Annual Meeting. The program of this annual conference is designed to acquaint members with various phases of food production, processing and distribution, and to keep them abreast of current trends and technological developments. The speakers invited are those whose subjects will build a well-rounded program, of interest to the greatest number. Annual conferences are highlighted by exhibits of new equipment, processes and special ingredients.

## AWARDS

The society administers the following awards:

**NICHOLAS APPERT AWARD** (Medal and $1,000). Each year the Institute makes this award to a person whose contributions to the food industry make him distinguished and outstanding in the opinion of the national jury.

**BABCOCK-HART AWARD** ($1,000). The purpose of this award, made by the Nutrition Foundation, is to encourage contributions to Food Technology which will have an influence on the nutritional well-being of the general population.

**FLORASYNTH FELLOWSHIP AWARD.** $1,000 granted annually to a young man or woman under 35 years of age, engaged in senior undergraduate or graduate work, as a candidate for an advanced degree, in recognized educational institutions in the United States or Canada, which is conducting fundamental investigations for the advancement of Food Technology.

**GENERAL FOODS FELLOWSHIP AWARDS.** Three Fellowships will be granted annually—no more than two to the same educational institution for the same calendar year, but will be renewable upon application to and approval by the Awards Committee of the Institute for a maximum of three years, in order to provide for continuity of studies leading to a doctorate degree, if the candidate so plans. However, candidates who plan only a year of research after obtaining the Bachelor Degree, or who plan to read for a Master's Degree, will not be excluded from these fellowship grants. Each Fellowship is worth approximately $4,000.

**GERBER'S INSTITUTE OF FOOD TECHNOLOGISTS UNDERGRADUATE AWARDS.** Six undergraduate Scholarships at $1,000 each, per year, intended to cover the last two school years leading to a Bachelor's degree in Food Technology, Food Engineering, or Food Science.

**THE SAMUEL CATE PRESCOTT FELLOWSHIP.** An engrossed plaque and $1,000 granted annually to an outstanding young man or woman for graduate studies in the general field of food science and food technology, at a recognized educational or research institution in the United States or Canada. The award is for one year and is not renewable.

**DODGE AND OLCOTT SCHOLARSHIP.** One renewable Undergraduate Scholarship each year at $1,000 and an engrossed plaque. To encourage Junior or Senior students in recognized institutions of the U.S.A. or Canada to take undergraduate work leading to a Bachelor's Degree in Food Technology, Food Engineering, or Food Science.

**ALEXANDER E. KATZ MEMORIAL SCHOLARSHIP.** $1,000 annually and an engrossed plaque to a deserving and outstanding undergraduate. Designed to encourage these students to take courses leading to a Bachelor's Degree in Food Technology, Food Engineering or Food Science.

The Institute also provides ten $300 scholarships to deserving young college students interested in the field of food technology.

(Continued from page 16 preceding technical section)

Philadelphia Department of Public Health in helping teach the City sanitarians the essentials of food technology. A one week course was held during the week of October 17th.

### FOOD REGULATIONS AND STANDARDS DISCUSSED

Program Chairman, Robert Lawhorn, introduced the speakers of the evening:

Robert C. Stanfill
Director of the Philadelphia Dist.
U. S. Food and Drug Admin.

Delmar K. Myers
Acting Director
Penna. Bureau of Foods & Chemistry

Dr. Morris A. Shiffman, D.V.M.
Chief, Milk and Foods Sect.
Philadelphia Dept. of Public Health

Henry S. Kushlan
Processed Products Standardization and Inspection
Fruit and Vegetable Branch
Agricultural Marketing Service, U.S.D.A.

D. P. Weber, Area Supervisor
Dairy Product Inspection and Grading Branch
A.M.S., U.S.D.A., New York City

Dr. Robert J. Lee, Head
Regulatory Section, Inspection Branch
Poultry Division
A.M.S., U.S.D.A., Washington, D. C.

Dr. M. W. Cohen
Inspector in Charge, Philadelphia Area
Agricultural Research Service
Meat Inspection Division, U.S.D.A.

Dr. John J. Powell, Chief
Standards Development and Inspection Unit
Bureau of Commercial Fisheries
Fish and Wildlife Service
U.S. Dept. of the Interior
Washington, D. C.

These speakers discussed the areas of the food industry in which they were interested and noted new regulations and standards. They also discussed ways they serve both the public and the food industry.

—*Murray I. Cooper*

## FUTURE MEETINGS FOR FOOD TECHNOLOGISTS

| | |
|---|---|
| **1961** | |
| February 2-3 | Statistical Quality Control and Instrumentation Workshop for the Food Industry (co-sponsored by Institute of Food Technologists, Western New York Section, and N.C.A.) Cornell University, Ithaca, New York. |
| February 28 –March 1 | Ninth Annual National Dairy Engineering Conference (co-sponsored by the Department of Agricultural Engineering and the Department of Food Science, Michigan State University, and the National Association of Dairy Equipment Manufacturers) Michigan State University, East Lansing |
| April 4-6 | Fifteenth Annual Meeting of the Research and Development Associates, Food and Container Institute, Hotel John Marshall, Richmond, Virginia |
| **May 7-11** | **Twenty-first Annual Meeting of the Institute of Food Technologists, Hotel Statler, New York, New York** |
| May 15-18 | Fourth International Congress on Canned Foods, Berlin, Germany (for information, address M. Henri Cheftel, J. J. Carnaud & Forges de Basse Indre, 71, Avenue Edouard Vaillant, Billancourt (Seine), France. |
| **1962** | |
| **June 10–14** | **Twenty-second Annual Meeting of the Institute of Food Technologists, Fontainebleau Hotel, Eden Roc Hotel, Miami Beach, Florida** |
| **1963** | |
| **May 19–23** | **Twenty-third Annual Meeting of the Institute of Food Technologists, Pantlind Hotel, Civic Auditorium, Grand Rapids, Michigan** |

An open invitation is extended to readers of FOOD TECHNOLOGY to send in to the Editorial Office, P. O. Box 164, Davis, California, notices of annual or national meetings of interest to food technologists.

# GEORGIA

### STUDENT CHAPTER OF IFT

**Get-acquainted mixer for new Food Technology majors sponsored by University of Georgia student chapter of IFT. Approximately 65 persons were in attendance, consisting of 45 Food Tech. majors, their wives, and faculty members and their wives.**

**Scholarship holders in Food Technology, 1960–61, University of Georgia. Left to right: John Galbreath, IFT Gerber scholarship; Rebecca Wheeler, University of Georgia freshman scholarship; Donald Downing, Refrigeration Research Foundation fellowship; Ted Dornseifer, National Science Foundation fellowship; and Susan Tilghman, Kroger freshman scholarship.**

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### IN MEMORIAM

The death occurred at Cambridge, England, on 2nd October 1960 of **Dr. Jack Brooks,** O.B.E., at the age of 58, Principal Scientific Officer at the Low Temperature Research Station. Dr. Brooks was in charge of the Eggs and Poultry Section.

### PERSONNEL

The Tranin Egg Products Company announces that **Dr. O. J. Kahlenberg** has joined the Tranin staff as Director of Sales and as Technical Director. Dr. Kahlenberg, one of the nation's outstanding authorities on eggs and egg products has most recently been Professor of Poultry Husbandry at Missouri University. Prior to that, he was Director of Research and Products Development for the F. M. Stamper Co., Moberly, Mo. From 1947 through 1950 Dr. Kahlenberg was Director of Research for the National Egg Products Association. From 1942 to 1947 he was Chemist-in-Charge of the National Laboratory of the Dairy and Poultry Inspection and Grading Division of the U. S. Department of Agriculture. Stationed in Chicago, he headed a large staff and was technical advisor for branch laboratories at Seattle and San Francisco. Earlier employment was with the Borden Biological and Chemical Laboratories in New York and with the Pennsylvania State University Nutritional Research Staff.

Dr. Kahlenberg received his B.S. degree in Agriculture at the University of Wisconsin, his M.S. degree at Rutgers University and his Doctorate from Cornell University. He is a member of various professional societies in his field and is the author of some 50 published scientific and non-technical papers.

**James Edward Welch** and **William L. MacNevin** were both recently promoted within the Wm. Underwood Co. Mr. Welch has

been appointed supervisor of Product Development and Quality Control, and Mr. MacNevin has been named chief engineer.

A graduate of the University of Maine, and with a master's degree from the University of Wisconsin,

J. E. Welch

W. L. MacNevin

Welch began his career with American Can Company in 1951. In 1955, he joined the University of Maine's Experimental Station, and two years later was appointed to the staff of the Maine Sardine Industry Research and Quality Control Laboratory at Bangor, Maine.

Mr. MacNevin is a graduate of Northeastern University, and has been employed for the past ten years by Underwood in production and engineering capacities.

**Dr. Arthur N. Prater,** president of the Gentry Division, Consolidated Foods, was honored recently by the Southern California Section, Institute of Food Technologists, by being elected Food Man of the Year.

A. N. Prater

Each year the 300 food technologist members of the IFT in the Southern California area honor an individual who has made outstanding contributions professionally toward the advancement of the food industry.

Dr. Prater chose "Food in a Changing World" as the subject of a talk he gave at the Rodger Young Auditorium, Los Angeles. His topics included the changing picture in processed, ready-to-eat foods and how homemakers have been saved hours of drudgery by today's instant milk, coffee, potatoes and onions, and by such other convenience foods as canned foods and frozen TV dinners.

**Dr. C. H. Bailey,** Charter Professional Member of the IFT and Dean Emeritus of the Institute of Agriculture, University of Minnesota, has been selected as the 1960 winner of the Charles F. Spencer Award. Given for outstanding achievement in the field of agricultural and food chemistry, the award includes a medallion and a $1,000 honorarium. It was established by the late Kenneth A. Spencer, founder of Spencer Chemical Company, in memory of his father and is administered by the Kansas City Section of the American Chemical Society.

A native of Minnesota, Dr. Bailey received his Ph.D. from the University of Maryland with a major in plant physiology. His master's degree was received at the University of Minnesota and his bachelor's degree from North Dakota Agricultural College. Dr. Bailey joined the staff of the University of Minnesota in 1913 as an assistant chemist. By 1920, he had been named as a professor. He became Dean and Director of the Department of Agriculture in 1942, holding this position until 1952. Dr. Bailey's prominence as an educator and chemist is well recognized in other countries, having served as Agricultural Research Training Consultant to the American Mission for Aid to Greece and as a Technical Consultant to the Foreign Agricultural Service, USDA. He is presently serving in an agricultural advisory capacity for the Seoul National University of Korea Cooperative Project.

C. H. Bailey

In addition to being the 1946 Nicholas Appert Medalist, Dr. Bailey has received the Osborne Medal for distinguished contributions in cereal chemistry (1932), and the Neumann Medal, awarded by the German Association of Cereal Research (1955). He has been named as "One of the Ten Ablest Agricultural and Food Chemists in the United States" (Chicago Section, American Chemical Society, 1947) and as "One of One Hundred Living Great of Minnesota" (Junior Chamber of Commerce, 1949).

**Mr. R. A. Hull** has recently been appointed as the eastern states representative Pacific Packtron Systems, Inc., Belmont, California, distributors of Illumatronic Checkweighers. Mr. Hull was formerly associated with Container Corporation of America as a Packaging Engineer. He is a graduate of Long Island Agricultural and Technical Institute.

R. A. Hull

**Mr. A. A. Winston** has been named research manager for the Perkins-S.O.S. division of General Foods Corporation. He joined General Foods in 1941 as a research chemist in the company's former Central Laboratories, and since 1954 has been research manager for GF's Franklin Baker operation in Hoboken, N. J. The Chicago-based Perkins and S.O.S. divisions were consolidated July 1 to form the Perkins-S.O.S. division, which

A. A. Winston

**FORMULA FOR PROFITABLE OPERATION**

# FOOD DRYERS by SARGENT

Generally, there are three approaches by a company to the selection of a process dryer — the engineering, the economic, and the important evaluation of the intangibles. We firmly believe that SARGENT will always be found on top from every approach, for many reasons, an important one being that our machines and our people consistently deliver as promised, and more. This is the SARGENT:

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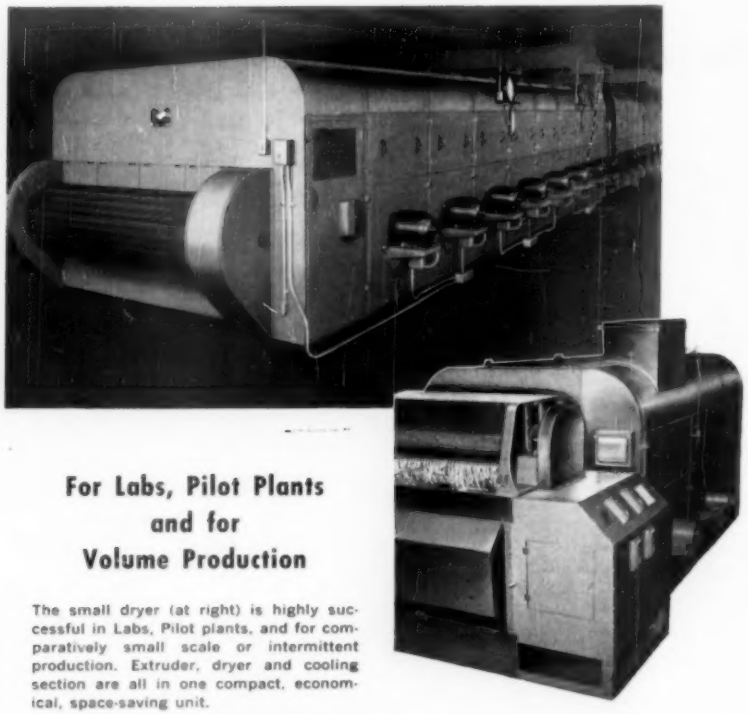

**For Labs, Pilot Plants and for Volume Production**

The small dryer (at right) is highly successful in Labs, Pilot plants, and for comparatively small scale or intermittent production. Extruder, dryer and cooling section are all in one compact, economical, space-saving unit.

The 14-section, 2-stage dryer (above) is engineered for high tonnage per hour production. Extruder, or granulator, together with baking and curing and cooling sections may be added by incorporating the same housing, where desired. Heat source for any SARGENT may be gas, steam or electricity. Airflow may be up, down, or in combination.

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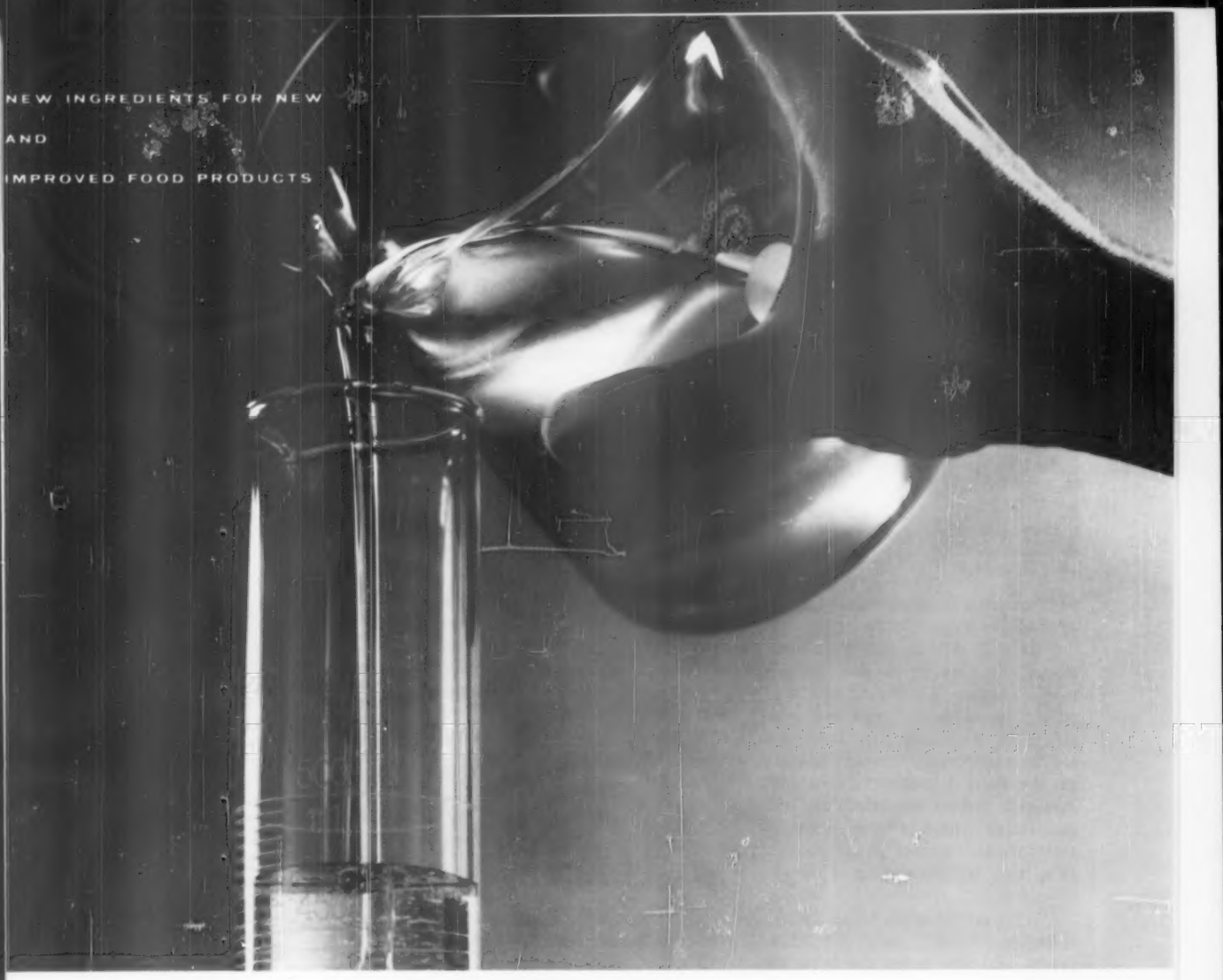
manufactures and markets salad dressing mixes and soft drink mixes.

**Mr. Samuel Cohen** has been appointed vice president in charge of sales of Glyco Chemicals (a division of Chas. L. Huisking & Co., Inc.). Mr. Cohen, formerly marketing director of the division, joined Glyco Chemicals in 1945 and has served in various laboratory and production capacities since that time. He received a B.S. in chemistry from City College of New York, an M.S. in organic chemistry from New York University, and an LL.B. from the Brooklyn Law School.

Samuel Cohen

The appointment of **Louis Bartenbach** to the newly created position of experimental chef in the product development section of the General Foods Research Center was announced recently. Before joining General Foods, Mr. Bartenbach was food director and chief chef instructor at the Culinary Institute of America. Prior to this, he was an officer-instructor for the food service department at Quartermaster School at Ft. Lee, Virginia. He also worked eight years as research chef with the H. J. Heinz Co. Mr. Bartenbach was educated here and abroad. In the U. S., he attended the Universities of Pittsburgh and Virginia, and the Richmond Professional Institute.

**Dr. Maurice Bender** has been appointed chief of the Research and Training Grants Branch in the Division of Air Pollution of the U. S. Public Health Service, Washington, D. C. He was formerly executive secretary of the Cancer Chemotherapy Study Section, Division of Research Grants at the National Institutes of Health, Bethesda, Md.

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COMMENT

Formation of a Food Division of United States Testing Company, Hoboken, New Jersey, to provide broad and complete testing, research and development, analysis and product evaluation services for the food, beverage and confection industries has been announced by A. L. Brassell, President. **Dr. Dean Foster**, international authority in the field, has been appointed manager of the new division.

**Dr. Dean Foster**

"United States Testing Company, recognized as the world's most diversified independent laboratory, is already supplying considerable assistance in food research and testing to many clients in the food industry," said Mr. Brassell. "The new division will coordinate these efforts, expand and broaden special programs for this, the nation's largest single industry.

"With stiffer competition facing them each year, food manufacturers and processors are devoting more and more time to research and development of new and better food products. Food additive problems are also increasing. Companies are insisting on objective data to guide their decisions, seeking all available information about the product itself, as well as marketing and consumer reaction to the product. United States Testing experience and organization can prove extremely helpful to the manufacturer, processor, distributor or seller of foods or beverage products, as well as their suppliers, trade associations and related groups."

A team of fifteen specialists has been assembled by United States Testing to operate the new division, with extensive experience in chemistry, microbiology, engineering, psychology and market research. Company facilities are equipped with highly advanced instrumentation for carrying out a complete program for the food industries, from product conception to nationwide marketing studies. The company will contract for all or a part of any such program, depending on client need. All information and resulting data is kept in strictest confidence.

Materials, products and processes which will come under the new division's capabilities are: edible or potable agricultural or chemical raw materials; formulations; processing equipment and effects; packaging materials and designs; flavor, color and extending additives; processing and preserving agents; pesticides and anti-microbial agents, and all conditions affecting final quality and shelf life.

For consumer product evaluation, U. S. Testing Company has standardized a method involving the study in sequence of six qualities: safety, durability, effectiveness, esthetics, economics, and special features.

# ABSTRACTS

**Selected by Norman H. Ishler**
**General Foods Research Center**

## ANALYTICAL METHODS

**Gas-chromatographic investigation of alcoholic beverages.** (In German).

MECKE, R. AND DE VRIES, M. *Z. Anal. Chem. 170*, 326–32 (1959).

A procedure is described for the sepn. and identification of the aroma-producing compds. in wines and spirits. These are extd. from 300 ml. of the sample with a mixt. of diethyl ether and n-pentane (2:1). Traces of EtOH are removed by washing with water and the ext. is dried over $Na_2SO_4$. The extractant is distd. off at 32° to leave a yellow oil (0.2 ml.). This is analyzed by vapor-phase chromatography.

**Automatic chloride analyzer.**

COULSON, D. M. AND CAVANAGH, L. A. (Stanford Research Inst., Menlo Park, Calif.). *Anal. Chem. 32*, 1245–7 (1960).

An automatic coulometric titration method has been developed for the detn. of chloride in combustible samples. The method is rapid, requires less sample, and is more accurate than methods currently used.

**Separation of sugars, amino acids and organic acids with the aid of ion-exchange resins.**

DILOV, K. AND GEORGIEV, D. *Compt. Rend. Acad. Bulg. Sci. 12*, 227–30 (1959).

An aq. soln. of the compds. is passed through a column of the cationite on which the amino acids are retained. The organic acids are retained when the percolate from the first column is passed through a column of the anionite. The percolate from the second column contains the sugars.

## BIOLOGICAL SCIENCES

### Biochemistry

**Structural studies of ribonuclease. III. A model for the secondary and tertiary structure.**

SCHERAGA, H. A. (Cornell Univ., Ithaca, N. Y.). *J. Am. Chem. Soc. 82*, 3847–52 (1960).

A three-dimensional model of ribonuclease has been constructed on the basis of available data on the primary, secondary and tertiary structure. The model will be useful for the design of future expts. to establish the complete structure of this protein.

**The effect of water upon the rate of heat denaturation of egg albumin.**

ALTMAN, R. L. AND BENSON, S. W. (Univ. of Southern California, Los Angeles). *J. Am. Chem. Soc. 82*, 3852–7 (1960).

The rate of heat denaturation of solid egg albumin has been found to be extremely sensitive to the water content of the protein. This sensitivity makes it extremely difficult to obtain precise rate data in the system. This is further complicated by the small amts. of hysteresis observed in the sorption process and the slow approach to sorption equilibrium.

**Inhibition of oxidation of ascorbic acid by ethylenediaminetetraacetate (EDTA).**

RAO, M. V. L., ET AL. *J. Sci. Food Agr. 10*, 436–41 (1959).

Small amts. of EDTA added to acidified solns. of ascorbic acid completely protected the vitamin against Cu-catalyzed oxidation for more than 6 days at room temp. At higher pH, EDTA acted as a prooxidant in proportion to its concn. The stability of the vitamin depended on the nature of the acid used, and loss of ascorbic acid with time was observed in solns. of oxalic acid and, to a lesser extent, $Cl_3CCO_2H$, even in the presence of EDTA. Under optimal conditions it was possible to heat solns. of ascorbic acid contg. EDTA on a water bath to complete evapn. without loss of vitamin.

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This new member of Givaudan's flavor family exactly reproduces the mouth-watering goodness of rich, ripe peaches. All the delicate nuances of aroma and flavor are captured and concentrated for use in your ice creams, sherbets, candy, pie fillings and pharmaceutical products. Our peach flavor is also recommended as a modifying note for orange or cherry beverages, and is available in powdered or liquid form. To achieve the full natural flavor of fresh peach in your product, try Givaudan's Imitation Peach. Write for samples and full information.

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**A model for the myosin molecule.**

KIELLEY, W. W. AND HARRINGTON, W. F. (Natl. Insts. of Health, Bethesda, Md.). *Biochim. et Biophys. Acta 41*, 401–21 (1960).

The molecular wt. of myosin has been detd. in 0.5 *M* KCl and 5 *M* guanidine · HCl. Utilizing these molecular wt. detns. and available X-ray diffraction, light scattering and optical rotatory data, a model for the myosin molecule is proposed, based on three equal wt. polypeptide chains in the form of a three-stranded α rope.

**A chemical method for the degradation of carbohydrates.**

KOHN, P. AND DMUCHOWSKI, B. L. (Univ. of Chicago, Ill.). *J. Biol. Chem. 235*, 1867–70 (1960).

A sequence of reactions is described by means of which all the carbon atoms of glucose may be individually isolated as barium carbonate. The nature of the reactions permits their use with a variety of other carbohydrates.

## Microbiology

**Variable factors in the new test for penicillin in milk.**

WITTER, L. D. AND TUCKEY, S. L. (Univ. of Illinois, Urbana). *J. Milk Food Technol. 23*, 230–3 (1960).

This study was undertaken to evaluate the results obtainable by the Arret and Kirschbaum assay for penicillin in milk, when the temp. for storing plates, time of storing plates, temp. for the test, and time of reading the test were modified. No evidence was found to support the recommendation that plates be stored at refrigeration temps. for not less than 3 days, no more than 5, before being used to perform penicillin assay. Changes are proposed to make the Arret and Kirschbaum method less restrictive, and more applicable to dairy laboratory procedures.

**Bacteria in electronically cooked foods.**

DESSEL, M. M., BOWERSOX, E. M. AND JETER, W. S. (State Univ. of Iowa, Iowa City). *J. Am. Dietetic Assoc. 37*, 230–3 (1960).

The effect of electronic vs. conventional cooking on the destruction of 4 representative bacterial species artificially inoculated into three foods is compared. Although requiring cooking periods ½ to ⅒ as long as conventional methods, electronic cookery was slightly more efficient in destroying the bacterial species used, except for the sporeformers subjected to over 30 min. of conventional heating in baked custard.

## Nutrition

**The response of man to dietary cholesterol.**

BEVERIDGE, J. M. R., ET AL. (Queen's Univ., Kingston, Can.). *J. Nutrition 71*, 61–5 (1960).

Ninety-three university students were placed upon a homogenized fat-free diet for a period of 8 days at which time they were divided into 8 groups and given a ration modified by the substitution of a butter-fat fraction, low in cholesterol, for 30% of calories at the expense of carbohydrate. Cholesterol supplements varying from nil up to 1600 mg./950 Cal. were added. Between intakes of 13 and 634 mg. cholesterol/day, the serum cholesterol increased sharply but no further significant increases were obtained at daily intakes of 1300 to 4500 mg.

**Amino acid requirements of children.**

NAKAGAWA, I., TAKAHASHI, T. AND SUZUKI, T. (Inst. of Public Health, Tokyo, Japan). *J. Nutrition 71*, 176–81 (1960).

On the basis of N balance, it has been estd. that the school children require 2 or 3 times as much essential amino acid mixt. as adults, if the pattern of Rose et al. for essential mixt. is not unbalanced for children. The total N is not sufficient at a level below 10 g. for children 12 to 14 years old.

**Evaluation of protein in foods. VI. Further factors influencing the protein efficiency ratio of foods.**

MIDDLETON, E. J., MORRISON, A. B. AND CAMPBELL, J. A. (Food and Drug Labs., Dept. of Natl. Health and Welfare, Ottawa, Can.). *Can. J. Biochem. Physiol. 38*, 865–9 (1960).

The protein efficiency ratio (P.E.R.) for casein found with a diet contg. 10% protein and 10% fat was slightly greater than that found with a diet contg. 15% protein and 20% fat, approximating the levels found in the "average" Canadian diet. The value for plant protein was similar under both conditions. Efficiency of carcass protein synthesis was much greater in animals fed casein than in those fed plant protein. With both sources of protein, the efficiency of carcass protein synthesis was similar with diets contg. 10% protein and 10% fat to those with 15% protein and 20% fat. It was concluded that P.E.R. detns. carried out on a 10% protein diet furnish a valid estimate of the nutritive value of protein and have several practical advantages.

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Produced by a unique process, Maggi's bulk hydrolysates are completely dependable – always uniform and unchanged by processing methods – and lack the typical flavor normally associated with hydrolyzed plant proteins.

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## M.I.T. Food Technology Department to Be Revamped

The Massachusetts Institute of Technology will create an augmented department, to be named the Department of Nutrition, Food Science and Technology. The enlarged department will be formed around the existing Food Technology Department. According to M.I.T. President Julius A. Stratton, this reorganization will offer greatly extended curricula and research activities in order to place more emphasis on the basic science and biological aspects of the vital area of food nutrition.

Named to head the department is Dr. Nevin Stewart Scrimshaw, director of the Institute of Nutrition of Central America and Panama (INCAP) and regional adviser on nutrition of the Pan American Health Organization, Regional Office for the Americas of the World Health Organization. Dr. Scrimshaw will assume his duties at M.I.T. as professor of nutrition and head of the department on August 1, 1961. Executive officer of the new department will be **Dr. Samuel A. Goldblith,** who has served as acting head since the death of Dr. Bernard E. Proctor.

According to Dr. George R. Harrison, Dean of the School of Science, plans for the department call for increased emphasis on food science and basic research oriented toward problems of human nutrition. He also states that the new program represents a fairly radical change in the work of the department. This means both an extension into new fields and strengthening of the food technology program in the direction of more basic research, particularly in the area of food toxicology and the effect of food processing on its nutritive value. Another feature of the new program will be a cooperative relationship between INCAP and M.I.T. For M.I.T. students this will mean opportunities to pursue studies in association with INCAP.

## Industry News

A new chromatography developing chamber, CHROMATOBOX,® for which Research Specialties Co. is the exclusive U. S. distributor has recently been announced. Chromatograms 2″ wide by 17″ long are developed in the CHROMATOBOX, which has over-all dimensions of 3″ x 3″ x 2″ high. Two features account for the small size of the CHROMATOBOX: (1) The paper strip is held in a plastic coil about 1″ in diameter; (2) Developing solvent is contained in a compartment that eliminates need for separate trough and anti-siphon rods. The CHROMATOBOX is recommended for laboratories having a small volume of chromatographic work, for individual student instruction, for easy portability to lectures and demonstrations, and as auxiliary equipment for test strips in laboratories having larger chromatography equipment. Illustrated literature is available on request to: Research Specialties Co., 200 South Garrard Blvd., Richmond, California.

Simultaneous photometric readings of a sample stream through 3 different light channels are continuously charted in a new Recording Flow Photometer introduced by Research Specialties Co. The equipment is directly applicable to automatic amino acid analysis, and it is said to eliminate time consuming fraction collecting and analyses of individual fractions from ion exchange columns. It is likewise applicable to analysis and control of effluents from chromatography columns and of many continuous flow production processes. Interchangeable light filters permit reading of optical transmission throughout the range of visible light. Baselines are stabilized by electronic regulation of lamp voltages to ±0.1%. Drift of photocell response is prevented by thermostatic control of the photometer. For additional information, write Research Specialties Co., 200 South Garrard Blvd., Richmond, California.

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**COMPACT BOTTLE UNCASER**
Model H-300

Low-priced unit uncases wide range of bottle sizes at speeds as high as 300 bottles per min. No air or hydraulic connections needed. Used with any standard filing table. New 600 bpm unit will soon be available.

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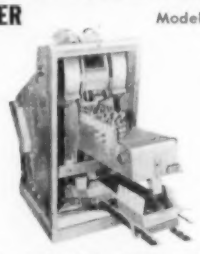

This high-speed unit handles a wide range of can, jar and bottle sizes (packed in single tier cases)—at speeds up to 30 cases per minute. No changeover necessary to switch from cans to bottles of same dimension.

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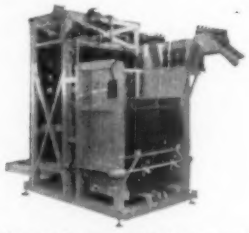

Straight-stack all standard cases and bottle sizes without mechanical adjustment, at speeds up to 30 cases per min. Designed for 36″ x 36″, 36″ x 48″, and 36″ x 60″ standard pallets. Can be furnished for other sizes. Available individually, or in combination.

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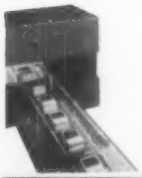

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This new low-cost RCA Electronic Metal Detector is designed specifically to meet the requirements of the food industry. It is highly sensitive to metallic particles, *non-magnetic or magnetic*, yet is ruggedly built. The waterproof aperture of the Metal Detector can be hosed down without damaging the circuitry. Bi-sectional construction simplifies installation around your conveyor line.

Five standard aperture sizes are available for inspecting bulk or packaged products at conveyor speeds of 15 to 600 feet per minute. Tramp metal—even as small as five hundredths of an inch in diameter—is readily detected.

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**Unless otherwise specified, particulars on the following positions may be obtained by writing, including the company name of your current employer: Executive Secretary, Institute of Food Technologists, 176 West Adams Street, Chicago 3, Illinois. Request by box number.**

WANTED: Quality Control Manager Trainee for frozen bakery product company with national distribution. Relocation probable. Personnel policies excellent. Experience in bacteriology, chemistry, dairy, egg and dairy products desirable. Indicate salary requirements and send résumé to BOX 821.

Excellent opportunity for Food Technologist—technical sales service work supporting national industrial sales organization. Should have B.S. in Food Technology or equivalent with experience in Production, Quality Control or Technical Sales. REPLY BOX 822.

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We are a major progressive Chicago based supplier of basic ingredients to the food industries. We require a competent analytical chemist with a thorough training in modern instrumentation who will apply his experience to many analytical problems associated with foods. Please send detailed background in full confidence. REPLY BOX 820.

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Section head, frozen foods. East coast location. B.S. or M.S. in food technology or chemistry with 3 years frozen food development laboratory experience, to head frozen food section of development laboratory. REPLY BOX P-667.

Process engineer. Midwest location. B.S. in food engineering or related field with three years mechanical design and process development in food industry. Will develop pilot plants and write process specifications. REPLY BOX P-611.

Research chemist. Eastern location. B.S. or M.S. in food technology with 0–2 years experience, to conduct applied research in laboratory and pilot plant. REPLY BOX P-614.

Associate researcher engineer. West coast location. B.S., M.S. or Ph.D. chemical or mechanical engineer, to work on problems related to food processing, materials handling, and packaging. REPLY BOX P-617.

Associate researcher. West coast location. B.S. or M.S. in chemistry or chemical engineering, to work on problems in product, process, and nutritional research of foods. REPLY BOX P-629.

Food technologist. Midwest location. B.S. in food technology or chemistry, for three year training program leading to position as technical sales service representative. REPLY BOX P-632.

Food technologist. Midwest location. Degree required for product development, quality control and production supervision. REPLY BOX P-671.

Chemist. Northeast location. Research and product development in fats, oils, emulsifier and baking field. Strong scientific background necessary. REPLY BOX P-676.

Baking technical and sales trainee. Northeast location. Scientific background required to prepare for selling career in baking field. REPLY BOX P-677.

Quality control manager. West coast location. College degree and quality control supervision experience required for direction and supervision of quality control department. REPLY BOX P-678.

Technical service representatives (3). Midwest location. Degree in food technology, chemical engineering, chemistry, with 0–2 years experience. Customer contact work with product technology, complaints, etc. REPLY BOX P-679.

Chemist. Midwest location. B.S. or M.S. in food technology, biochemistry or related field, for product development and applied research on nutritional specialty products. REPLY BOX P-681.

Food technologist. Eastern location. B.S. in food technology or chemistry, for applied research on canned foods and special problems of interest to canning industry. REPLY BOX P-682.

Product control technologist. East, midwest, west, location. B.S. or M.S. in food technology or related sciences, for quality control, specifications, and technical service. REPLY BOX P-684.

Food technologist. Midwest location. B.S. in chemistry plus M.S. in food technology preferred for project leader in product development. Experience not required. REPLY BOX P-685.

Home economist. Midwest location. B.S. and/or M.S. in home economics or food technology desired from an individual who will supervise and administer kitchen function for developing tests to study new products. Ability to analyze results and write comprehensive reports. REPLY BOX P-686.

Research Chemist. West coast location. A.B. degree required, for person to develop new uses for algin or similar hydrophilic colloid products. REPLY BOX P-687.

Midwest location. College graduate with scientific degree and 3–4 years sanitation and/or statistical quality control work desired to assist in direction of quality control program. REPLY BOX P-690.

Customer Research Trainee. B.S. with up to two years experience in research or technical service, for position involving service to metal container packaging industry. REPLY BOX P-692.

Staff Food Technologist. Midwest location. Man with B.S. in food technology or equivalent, and production, quality control or technical sales experience, particularly in the baking or allied industries. Position includes combination of control staff and field work on problems related to use of special salt products. REPLY BOX P-694.

Production trainee. Western location. B.S. or B.A. in food technology or related fields required for above position in fruit and vegetable canning operations. REPLY BOX P-700.

Instructor in research. Midwest location. Background in chemistry, bacteriology, or statistics necessary to work in quantity food research program in a College of Home Economics. REPLY BOX P-701.

Food Technologist. West Coast location. Degree in food technology with a number of years experience in: product development, general sanitation control, formulation, quality control, freezing effects study, production flow, equipment research, trouble-shooting on flavors, colors and textures, and bacteria studies. REPLY BOX P-703.

Western location. College degree and experience in fruit juices and flavors preferred. Administrative position in sales and research. REPLY BOX P-705.

Manager of Quality Control. Canadian location. University graduate with food laboratory experience necessary for man who is capable of establishing a laboratory and carrying out a quality control program. REPLY BOX P-707.

Analytical chemist. Midwest location. B.S. in food chemistry or analytical chemistry, to fill position involving general analytical chemistary as related to various types of food products. REPLY BOX P-711.

Food Technologist. Midwest location. B.S. in food technology or related areas required. Position entails food product development with all commodities. REPLY BOX P-712.

Food technologist—cereal and baked products. Midwest location. B.S. with some graduate work completed. Product development work on cereal products for armed forces rations. REPLY BOX P-713.

Compounder-flavorist. West coast location. Minimum education of B.S., plus experience in flavor compounding and aromatics blending. Position entails compounding and formulation of flavoring materials. REPLY BOX P-714.

Technical sales. West coast locations. B.S. degree or better, plus sales experience or experience in use of flavors and flavored products. Technical assistance to the customer, and sales, both involved. REPLY BOX P-715.

Food Technologist. Midwest location. College graduate, preferably with an M.S. in food science or technology needed to assist in the biological research area, including food processing techniques and equipment. REPLY BOX P-718.

Chemist. Midwest location. Recent graduate with a B.S. in chemistry or food technology. Duties include examination of cereal products, spices, instrumental analysis, meat products, and food chemistry. REPLY BOX P-721.

CHEMIST with a B.S. degree, with a background in the use of essential oils and oleoresins. This is not a run-of-mill opportunity. It is first class in every respect and has a continuing challenge for a live-wire who is able to help analyze, duplicate and develop flavoring for the meat processors. We have all the standard forms of security such as pension, bonus, periodic increases, etc., etc. Our plant is located in the midwest and we are one of the large companies in our industry. Working facilities and surroundings are excellent. Salary commensurate with ability and background. All replies will be held in strictest confidence. REPLY BOX 823.

RESEARCH KITCHEN
PRODUCT DEVELOPMENT

Leading national supplier of basic ingredients to the food industry has excellent opportunity in its Research and Product Development kitchen. The company is located in Chicago. The applicant should have 10 years experience in meat processing including curing, smoking, sausage manufacturing, and related meat areas. Should have enough supervisory and organizing experience to direct Research and Development activities. Should be creative and research minded. Excellent salary for the man with the proper qualifications. Reply in detail stating experience and salary required. REPLY BOX 824.

## SITUATIONS WANTED

**Unless otherwise specified, particulars on the following individuals may be obtained by writing: Executive Secretary, Institute of Food Technologists, 176 W. Adams St., Chicago 3, Ill. Request by box number.**

AVAILABLE: Food Technologist, B.S. Purdue 1948. Some graduate work. Experience—canning, carbonated beverages and dairy products. Desire Chicago location. REPLY BOX 762.

AVAILABLE: CEREAL CHEMIST and FOOD TECHNOLOGIST—twenty years of varied and responsible exp. in products control, research and development—excellent background in commercial baking and baking technology—seeks promising position in Product Development and/or Technical Service. REPLY BOX 819.

Recent chemistry-biology graduate desires laboratory technician or chemist position. Her major experience consists of five years in quality control. Salary, $4,200. REPLY BOX A-62.

Production position or technical service position desired by food technology graduate with background in confections. Salary, $6,900. REPLY BOX A-63.

Recent food technology graduate desires production, technical sales, or quality control position in the field of fruits and vegetables. Salary, $4,800. REPLY BOX A-64.

Management position in technical sales or production desired by individual experienced in packaging, research and engineering, and technical sales. Salary, $12,000. REPLY BOX A-71.

1957 chemistry graduate interested in cereals, baked goods, and fats and oils, desires production, technical service or quality control position. Salary, $7,200. REPLY BOX A-74.

Bacteriology graduate desires production or quality control position as food technologist to work with canned foods. Salary, $7,000. REPLY BOX A-77.

Individual experienced in soups, heat processed foods and frozen foods desires production or technical service position. REPLY BOX A-78.

Food technology graduate seeking quality control position preferably with canned fruits and vegetables. Also interesfed in product development and technical service. Salary, $7,200. REPLY BOX A-92.

Technical service position in the confections industry, preferably chocolate and sugars, desired by chemistry and marketing graduate. Salary, $7,000. REPLY BOX A-94.

Line or staff position in manufacturing, packaging or engineering desired by experienced factory management graduate. Salary, $10,000. REPLY BOX A-99.

Young food technology graduate desires position in food industry. REPLY BOX A-102.

Experienced packaging individual desires product development, production, or packaging research position which will allow independent operation. Salary, $10,800. REPLY BOX A-104.

Technical service or quality control position in the bakery or confectionery fields desired by fairly recent food technology graduate. Salary, $7,000. REPLY BOX A-105.

M.S. with experience in dairy, cereals, and beverages desires position similar to director of development and research. Salary, $15–20,000. REPLY BOX A-301.

Administrative position desired in area of product development, industry research or quality control by experienced M.S. Salary, $10,800. REPLY BOX A-302.

Research position, preferably with industry in the field of citrus products desired. Salary, $8,000. REPLY BOX A-306.

Product development, production, or research interest an M.S. food technologist with five years experience in these areas. Salary, $7,500. REPLY BOX A-310.

Research food technologist position desired by individual with recent M.S. food technology. Product preference includes meats, vegetables and fruits. Salary, $8,000. REPLY BOX A-311.

Individual with a physical chemistry degree plus an M.B.A., desires a management and administration position in the production field. Salary, $13,500. REPLY BOX A-315.

M.S. with experience in food manufacturing desires position as food technologist, bacteriologist, or in production. Salary, $8,500. REPLY BOX A-318.

Production or research position in the field of meats desired by recent M.S. graduate. Also interested in flavor and color. Salary, $8,500. REPLY BOX A-324.

Technical service position desired by individual interested in dairy products. Interest range also includes quality control and research. Salary, $8,000. REPLY BOX A-325.

M.S. in food science desires position as production manager. All experience has been in this area. Salary, $11,000. REPLY BOX A-326.

Position involving supervision of quality control or technical sales desired by food technology graduate. Product development position also of interest. Salary, $8,400. REPLY BOX A-327.

Fruits and vegetables interest individual seeking position as food technologist in either research or quality control. Salary, $6,000. REPLY BOX A-336.

Recent M.A. in microbiology desires research position in this area. Salary, $7,200. REPLY BOX A-328.

Experienced individual with M.S. in dairy mfg. desires line management or manager of product development position. REPLY BOX A-341.

New food technology M.S. with a Bachelors of Education in Home Economics, desires technical writing position in the area of product development, industry or academic research. She has one year experience in teaching home economics. Salary, $6,500. REPLY BOX A-342.

Another new food technology M.S. with a B.S. in bacteriology desires academic research and/or teaching position, with emphasis on microbiology. She has had two years experience in academic research. Salary, $6,000. REPLY BOX A-343.

Ph.D. food technologist desires research position. He has had two years experience in this field with particular application in the area of gas chromatography. Salary, $6,000. REPLY BOX A-605.

Young Ph.D. desires commercial research and development position which deals with poultry, eggs, or meats. Salary, $8,000. REPLY BOX A-606.

Northeast location desired by Ph.D. food technologist. Product development and industry research with sea foods are areas of interest. Salary $12,000. REPLY BOX A-607.

Food technologist with Ph.D. in that field desires product development or industry research position as a food chemist or food technologist. Salary, $7,000. REPLY BOX A-620.

Research or teaching position desired by individual with Ph.D. Product preferences include fruits, vegetables, coffee, tea, confections, carbonated beverages, and cereals and baked products. Salary, $9,000. REPLY BOX A-622.

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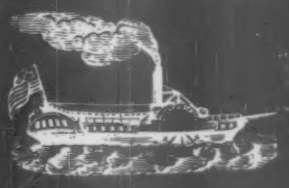
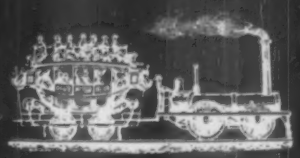


### PURE COFFEE CONCENTRATE

American Foods' **Pure Coffee Concentrates** are made by an **exclusive** process especially developed for ice cream flavoring. You get delicious, **rich** "fresh-brewed" **flavor** without any bitterness or fade-out.



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American Foods' **Vari-Top Sauces** are available in a wide variety of rich-tasting, **natural** flavors. (And for **perfect** uniformly distributed patterns, use American Foods' 6- or 8-spoke **Variegator.**)

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### FLAVORS, PUREES and EXTRACTS

American Foods' offers **true fruit extracts** and **purees** of "field-fresh" quality and highest concentration.

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- Strawberry
- Many others

### VANILLA

American Foods' **Vanillas** are known for their perfect **flavor balance.** You get finer, richer taste and **superior** flavorings that won't fade. You can rely on top **quality** Pure Vanilla Extracts, Fortified Vanilla Extracts or Vanilla Powders—also **custom-made** formulations.

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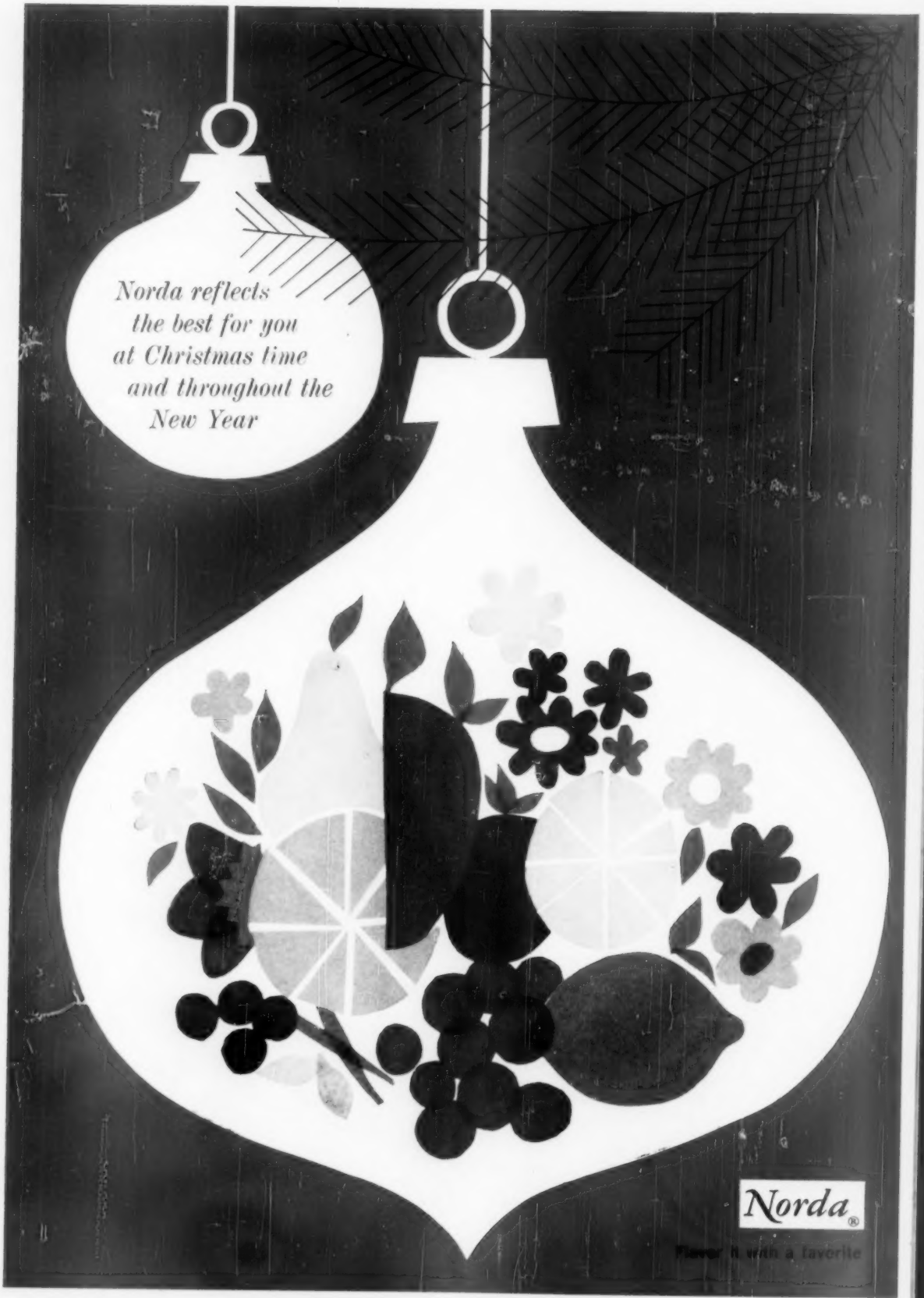
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